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OSMOTICALLY INDUCED MASS TRANSFER IN BIOLOGICAL SYSTEMS: THE SINGLE CELL AND TISSUE BEHAVIOR

by

Christian J. Toupin

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF Doctor of Philosophy
IN
Food Engineering

Department of Food Science

EDMONTON, ALBERTA
Spring 1986

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ISBN 0-315-30250-X

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OSMOTICALLY INDUCED MASS TRANSFER IN BIOLOGICAL SYSTEMS: THE SINGLE CELL AND TISSUE BEHAVIOR

DEGREE FOR WHICH THESIS WAS PRESENTED **Doctor of Philosophy**

YEAR THIS DEGREE GRANTED **Spring 1986**

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Aux auteurs de mes jours et à Françoise ...
pour ces longues années de séparation.

Abstract

The main emphasis in this work was placed on the development of a mathematical model incorporating cellular membrane characteristics for the description of water and solute(s) fluxes in complex cellular structures. In the study and development of the equations that describe the osmotic systems under consideration, the theory of irreversible thermodynamics was employed and the original or more fundamental form of phenomenological relations, namely the *Onsager fundamental form*, was preferred over the "practical" form used by most researchers.

Statistical evaluations of macroscopic permeability coefficients using a Modified Simplex method, based on modeling and simulations of osmotic dehydration of single cell suspensions, permitted characterization of cell membrane permeability properties. Studies such as the influence of temperature and concentration on the coefficients and consequently on the permeability characteristics of the cells were carried out.

Based on the theoretical formalism developed for describing osmotic dehydration of single cell suspensions, a complete model has been devised to depict the isothermal mass transport phenomena that occur in an organized arrangement of cells. This model is composed of two main parts: (1) the cell interactions, handled by the theory of irreversible thermodynamics, and (2) the overall diffusion process, simulated by second-degree Fickian-related equations. Owing to the complexity of biological tissue structure, a hypothetical cellular matrix made of equivalent cylindrical cells has been designed. The mathematical model tested on simple tissue geometry has been used to simulate movement of water, permeable and impermeable solutes in the tissue. Parametric studies permitted examination of the influence of various cellular and tissue properties on the dynamics of mass transport phenomena occurring in the cellular structures considered.

Acknowledgements

I wish to express my special appreciation to my supervisor, Dr. M. Le Maguer, for his counsel and encouragement throughout the course of this study. Special thanks are due to Dr. P. Jelen, Dr. L.E. McGann, Dr. J. Masliyah and Dr. E. Rotstein for helpful discussions and for their service as committee members. Special thanks are also extended to Dr. J.R. McGregor and Dr. J. Sheahan for introducing me to the realm of statistics.

I would like to express my gratitude to Mr. L. Steele, master of the keystroke and Textform wizard, for making that "monument" possible. My deepest indebtedness to Mr. J. Bourgois and Mr. G. Choudhury for those epic discussions and their untiring assistance. Sincerest thanks are also extended to all other members of the Department of Food Science at the University of Alberta, whose encouragement and friendship meant so much.

Finally, I gratefully acknowledge the financial support offered by the Natural Sciences and Engineering Research Council of Canada, the Alberta Heritage Foundation through the Farming for the Future Program and Agriculture Canada, which made completion of this project possible.

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List of Symbols

Unless otherwise stated, the equation number (Eq. #) given in parentheses after the description refers to the equation in which the symbol is first used or thoroughly defined. Units are given in the SI system (Systême International).

Chapters 2 to 5

Symbol	Description
a_k	thermodynamic activity of species k (dimensionless; Eq. 2.25)
A	membrane surface area (m^2; Eq. 2.14)
A_{kk}, A_{jk}	apparent transfer energies of species k and (jk)-couple for j≠k (J $kgmol^{-1}$; Eq. 3.7, 3.8)
C_k	concentration of species k (kgmol m^{-3}; Eq. 3.2)
$\bar{C}_j$	average concentration of species j across the membrane (kgmol m^{-3}; Eq. 2.15)
C_j*	reference concentration for species j (kgmol m^{-3}; Eq. 4.14)
F	Faraday constant (C $equivalent^{-1}$; Eq. 2.25)
J_j	molar flux of species j (kgmol m^{-2} s^{-1}; Eq. 2.13)
J_i	i-th generalized flux ([units of quantity transferred] m^{-2} s^{-1}; Eq. 2.1)
$\mathcal{J}_j$	modified transmembrane flux of species j (kgmol m^{-3} s^{-1}; Eq. 4.19)
J_v	volumetric flux (m^3 m^{-2} s^{-1}; Eq. 2.18)

L_{jk}	jk-th macroscopic phenomenological coefficient, Onsager form for isothermal systems (kgmol^2 J^{-1} m^{-2} s^{-1}; Eq. 2.13)
L_{ij}	Onsager coefficient, ij-th generalized phenomenological coefficient (K [units of quantity transferred]^2 J^{-1} m^{-1} s^{-1}; Eq. 2.4)
$\mathbf{L}_{ij}$	ij-th modified macroscopic phenomenological coefficient (kgmol m^{-3} s^{-1}; Eq. 4.17)
Lp	hydraulic conductivity, water permeability coefficient (m^3 N^{-1} s^{-1}; Eq. 2.18)
n_k	number of moles of species k (kgmol; Eq. 2.7)
$\dot{n}_k$	molar flow of species k, rate of appearance or disappearance of species k (kgmol s^{-1}; Eq. 2.7)
ϕ	normalization factor (J m^{-1} kgmol^{-1}; Eq. 4.17)
p_{kk}	macroscopic permeability coefficient of species k (kgmol N^{-1} s^{-1}; Eq. 3.5)
p_{jk}	macroscopic permeability coefficient of (jk)-couple (cross-coefficient) for $j \neq k$ (kgmol N^{-1} s^{-1}; Eq. 3.6)
P	pressure, hydrostatic pressure (N m^{-2}; Eq. 2.18)
$\dot{q}$	energy flow (J s^{-1}; Eq. 2.8)
$\mathcal{R}$	universal gas constant (J kgmol^{-1} K^{-1}; Eq. 2.25)
Si	internal entropy (J K^{-1}; Eq. 2.8)
T	absolute temperature (K; Eq. 2.8)
T*	reference temperature (K; Eq. 4.18)

$\bar{v}_k$	partial molar volume of species k (m^3 $kgmol^{-1}$; Eq. 2.19)
V	volume (m^3; Eq. 4.1)
V_s	apparent cellular solution volume (m^3; Eq. 4.8)
V_d	apparent cellular nonosmotic volume (m^3; Eq. 4.8)
X_j	j-th generalized differential thermodynamic force (J K^{-1} m^{-1} [units of quantity transferred]$^{-1}$; Eq. 2.1)
z_k	ionic valency of species k (equivalent $kgmol^{-1}$; Eq. 2.25)
θ	time (s; Eq. 2.7)
$\lambda_{(kj)}$	linkage coefficient ($kgmol^2$ J^{-1} m^{-2} s^{-1}; Eq. 2.21)
μ_k	chemical potential of species k (J $kgmol^{-1}$; Eq. 2.8)
ν_j	number of dissociation products related to species j (units $kgmol^{-1}$; Eq. 4.7)
π_t	osmotic pressure related to species t (N m^{-2}; Eq. 2.18)
σ_k	reflection coefficient of species k (dimensionless; Eq. 2.18)
ϕ_j	osmotic coefficient of species j (dimensionless; Eq. 4.7)
Φ	electrical potential (V; Eq. 2.25)
Ψ	dissipation function (J s^{-1}; Eq. 2.12)
ω_{kk}	permeability coefficient of species k (solute) (kgmol N^{-1} s^{-1}; Eq. 2.20)
ω_{kj}	generalized permeability coefficient of (kj)-couple (cross-coefficient) (kgmol N^{-1} s^{-1}; Eq. 2.20)

Subscripts, superscripts and special characters

[subscript]	related to the [subscript] species, unless otherwise specified
$^{o}\ ,\ _{o}$	indicates a reference quantity or a quantity related to such reference (Eq. 3.2, 4.11)
$_{(\ )}$	indicates an alternative subscript (Eq. 3.4)
$^{(1)},\ ^{(2)}$	related to phase (compartment) 1 and 2 (Eq. 2.8)
(eff)	indicates an effective (apparent) quantity (Eq. 4.7)
$x\,\infty$	value of x at equilibrium, asymptotic value (Eq 4.46)

Mathematical notation

d	differential operator, total derivative (Eq. 2.7)
∂	differential operator, partial derivative (Eq. 2.2)
Δ	difference operator, finite difference, i.e. quantity in phase 2 - quantity in phase 1 (Eq. 2.8)
Σ_i	summation operator (Eq. 2.2)
!	factorial operator (Eq. 2.2)
$\ln x$	logarithm of x to the base e (Eq. 2.25)
$\exp x$	exponential function of x (Eq. 3.7)
$\sqrt{x}$	square root of x (Eq. 2.27)

$\|_x$	evaluated under the x condition (Eq. 2.5)
$\|x\|$	absolute value of x (Eq. 2.27)

Statistical notation

k	number of replicate observations (Eq. 4.30)
m	number of replicated means (Eq. 4.37)
n	number of individuals in a given sample (Eq. 4.34)
σ^2	population variance, sigma squared (Eq. 4.28)
Var(x)	variance of the x-th population (Eq. 4.33)
W	weighting factor (Eq. 4.28)
z	standardized value of the dependent variable Z (Eq. 4.49)
Y , Z	dependent variable (Eq. 4.29, 4.49)
$\hat{}$	over a quantity, indicates a predicted or estimated value (Eq. 4.29)
$\bar{}$, $\bar{\bar{}}$	over a quantity, indicates, respectively, a mean and a grand mean (Eq. 4.30, 4.37)
$_i$	related to the i-th value of the independent variable(s) (Eq. 4.28)
$_j$	related to the j-th observation of a given sample of replicates (Eq. 4.30)
$_k$	related to the k-th individual of a given observation (Eq. 4.39)

Chapters 7 to 9 and Appendix 4

In order to avoid redundancy, the symbols adopted in Chapters 2 to 5 are preserved and are used to designate similar quantities in Chapters 7 to 9 and Appendix 4. The previous symbolism is however enlarged to encompass a broader context. For that purpose, a "*2-characters*" notation is employed and is devised as follows:

1. The *first* character describes the quantity to which the symbol is related (see preceding list). It is in *upper case* (for most symbols) or in *Greek characters*.

2. The *second* character indicates the part of the complex cellular structure (or its equivalent representation) which is designated by the preceding one. This second letter is *always* in *lower case* and is restricted to the following 7 characters:

"b" ≡ the *bath* (osmoticum) or *buffer cylinder*.
"c" ≡ the *cell* (cellular, volume delimited by plasmalemma).
"i" ≡ the *interstitium* (extracellular, apoplastic).
"m" ≡ the *cell membrane* (plasmalemma).
"p" ≡ the *plasmodesma pseudo-membrane* (symplastic).
"s" ≡ the *extracellular free space*.
"w" ≡ the *cell wall*.
" " ≡ refers to a *total* (global) quantity.

3. Supplementary characters, i.e. *subscript(s)*, *superscript(s)* or *special characters* may be added to detail the description further. Hence, under the preceding convention, the symbols "Vc'" or "$\bar{c}m_i$" are to be read, respectively, as: the *cellular* (c) *volume* (V) of the *tissue* ($'$) and the *average concentration* ($\bar{c}$) of *species i* ($_i$) across the *plasmalemma* (m).

The following list contains symbols particular to the aforementioned sections or redefined in view of a different use in these chapters. All symbols, unless otherwise specified, adopt the above notation.

Symbol	Description
a_k	k-th coefficient of a polynomial (dimensionless; Eq. 9.2)
A	surface area (m^2; Eq. 8.10)
CMM1, CM, CMP1, CSM2, CSM1, CB, CO, C1, C2	lumped nonlinear terms of finite-difference equations (Eq. A4.96, A4.100, A4.109)
d^1	diameter of sphere representing the total volume of the average unit cell (m; Eq. 8.17)
dc^1	diameter of sphere representing the unit cellular volume (m; Eq. 8.5)
đ_{ki}	generalized multicomponent diffusion coefficient for the pair k-i ($m^2\ s^{-1}$; Eq. 7.1)
D_i	diffusivity of species i in solution, solution diffusivity ($m^2\ s^{-1}$; Eq. 7.5)
$D_{i\infty}$	binary diffusivity of species i at infinite dilution ($m^2\ s^{-1}$; Eq. A4.29)
$D^{m\infty}$	geometrical mean of the binary diffusion coefficients at infinite dilution of the diffusing solutes present in the system, reference diffusivity ($m^2\ s^{-1}$; Eq. A4.28)
Đ_i	apparent pseudo-binary diffusivity of species i ($m^2\ s^{-1}$; Eq. 7.3)
Ɖ_i	corrected diffusivity of species i ($m^2\ s^{-1}$; Eq. 8.19)

$\underline{F}i_i$	reduced bulk apoplastic molar flux of species i (dimensionless; Eq. A4.130)
$Fo(m)_i$	Fourier number for mass transfer for species i (dimensionless; Eq. 8.19)
h	space increment (dimensionless; Eq. A4.79)
k	time increment (dimensionless; Eq. A4.80)
ℓ	length of the equivalent cylindrical unit cell (m; Eq. 8.17)
m	mass (kg; Eq. 8.106)
M_i	molecular weight of species i (kg kgmol^{-1}; Eq. 8.106)
Ni_{iN}	molar flux of species i in the interstitium with respect to a fixed frame of reference (kgmol m^{-2} s^{-1}; Eq. 8.61)
P	hydrostatic pressure in excess of the atmospheric pressure (N m^{-2}; Eq. 8.37)
R	cylinder radius (m; Eq. 8.24)
$\underline{R}_i$	reduced production-depletion term for species i (dimensionless; Eq. A4.34)
$\underline{R}v$	reduced volumetric production-depletion term (dimensionless; Eq. A4.37)
$v^{\blacksquare}$	volume average velocity (m s^{-1}; Eq. 8.72)
Vd	apparent cellular nonosmotic volume (m^3; Eq. 8.90)
z	direction of diffusion, space variable (m; Eq. 7.2)
αp	proportion of total cell membrane occupied by plasmodesmata (dimensionless; Eq. 8.10)

β_1	variation of the cell wall elastic modulus with respect to the intracellular hydrostatic pressure (dimensionless; Eq. 8.42)
β_2	value of the cell wall elastic modulus at incipient plasmolysis ($N\ m^{-2}$; Eq. 8.42)
ϵ	porosity or void fraction of a porous structure (dimensionless; Eq. 7.6)
κ	compliance factor (dimensionless; Eq. 8.14)
K_i	pseudo-binary mass transfer coefficient for species i ($m\ s^{-1}$; Eq. 8.115)
λ	acceleration factor (dimensionless; Eq. A4.127)
ξ	cell wall elastic modulus ($N\ m^{-2}$; Eq. 8.41)
τ	tortuosity of a porous structure (dimensionless; Eq. 7.6)
T_0	reference thickness at full turgor (m; Eq. 8.114)
ϕ	volume fraction (dimensionless; Eq. 8.1)
ψ	apparent diffusibility of a porous structure (dimensionless; Eq. 7.5)
A, B, Γ, T, E, Ψ	variables introduced to simplify writing of equations (Eq. A4.94, A4.105, A4.120)

Subscripts, superscripts and special characters

$_{i,k}$	indicates a quantity related to species i and k, unless otherwise specified (Eq. 7.1)

^{j}	indicates a quantity related to the j-th cell of a structure (Eq. 8.16)
^{t}	indicates a quantity related to the tissue (Eq. 8.1)
^{x}	indicates quantity related to the last cell of the column (Eq. A4.108)
^{1}	indicates a quantity related to the average unit cell (Eq. 8.3)
$^{-}, ^{+}$	quantity related to the (j,j-1)-th cell and (j,j+1)-th cell interaction, respectively (Eq. 8.50, 8.57)
$^{/}$	indicates a fractional part of a quantity (Eq. A4.10)
$^{(\ )}, _{(\ )}$	indicates an alternative super- or subscript (Eq. 8.57)
#c	number of cellular units in thickness (Eq. 8.113)
#c^{t}	number of constitutive average unit cells in tissue analog (Eq. 8.4)
#col^{t}	total number of columns representing the tissue under consideration (Eq. 8.106)
#grp	number of grid points in the cell (Eq. A4.79)
*	indicates a reference quantity (Eq. 7.4)
■	indicates a quantity related to the volume average velocity (Eq. 8.73)

Mathematical notation

div , grad	vector differential operator, "*nabla*" or "*del*" operator (Eq. 7.1)
erfc x	complementary error function of x: $1 - \text{erf } x$ (Eq. 7.4)
int x	integer part of x (Eq. 8.114)
$\min(x,y)$	the minimum value between the quantities x and y (Eq. 8.83)
Q	value of calculated integral (Eq. A4.128)
(avg)	indicates an average value for an interval of time $\Delta\theta$ (Eq. 8.61)
δx	indicates a small fraction of x (Eq. 8.63)
Δ	difference operator, finite difference, i.e. intracellular quantity - extracellular quantity (Eq. 8.38)
π	pi constant, 3.14159..., unless otherwise specified (dimensionless; Eq. 8.5)
Π	multiplication operator (Eq. A4.29)
$x \uparrow y$	indicates that x is to the power y (Eq. 8.43)
$\int$	integral operator (Eq. 8.81)
$\vert_x$	evaluated at x (Eq. 8.61)
$\vert_{in}$	evaluated at the interface between 2 cells, unless otherwise specified (Eq. 8.123)
$\vert_s$	evaluated at the surface of the tissue, unless otherwise specified (Eq. 8.106)

$\vert_{o}$	evaluated at the center of the tissue, unless otherwise specified (Eq. 8.120)
$\vert_{m}^{n}$	evaluated at the n-th time level and m-th grid point (Eq. A4.81)

1. INTRODUCTION

1.1 General background of the work

The phenomena involved in applied biological fields such as food processing and preservation or cryobiology are closely related to thermodynamics, transport processes, reaction kinetics, and other principles of engineering sciences. Traditionally, biological processes were approached on a relatively qualitative and empirical level. However, it is more and more recognized that the most needed advances require a more fundamental and quantitative understanding of underlying mechanisms. That is why for the past decade a new breed of "*bioengineers*" has slowly emerged in answer to the increasing demand for people able to bridge the gap between the biological world and the formalism of more basic sciences.

The present work is an example of such an effort where principles of the theory of irreversible thermodynamics, of transport phenomena, of numerical analysis and statistics were applied to study a biologically-oriented problem: the quantitative analysis of *osmotically induced mass transfer in biological systems*.

Along with an increase in interest in the function of biological membranes, there is a rapidly growing need for better qualitative and quantitative understanding of fundamental phenomena and mechanisms underlying mass transfer in complex cellular structures. Through a deeper comprehension, processes such as freezing and preservation of biological materials or transformation, preservation and storage of foods can be designed with greater confidence and reliability and more economical and energy-efficient techniques can be created.

Mathematical and computer simulations of various physical and chemical phenomena have recently become very common with the advent of high speed digital and analog computers. Although used quite extensively in fields such as chemical engineering, relatively very limited work has been done on the fundamentals of transport phenomena in biological tissues. This is partly due to the fact that people working in biological fields are traditionally less inclined to endeavor in the mathematical and numerical analysis necessary to carry out such simulations and are therefore reluctant to step into this alternative avenue. It would seem that, if ever *interdisciplinary* efforts were called for, it is here, in the area of the simulation of physiological and biological systems.

This work aims at contributing to this area through the following objectives:

1. Firstly, to review critically the use of irreversible thermodynamics in the derivation of *macroscopic* dynamic models for the description of passive transport of substances across cell membranes (*simple passive transport theory*);
2. Secondly, to present a novel approach to the description

of transport phenomena across biological membranes based on a more fundamental form of the relations derived from the theory of irreversible thermodynamics;

3. Thirdly, to discuss the statistical estimation of the permeability parameters contained in the generated equations with regards to the experimental techniques used;

4. Fourthly, to show how the proposed phenomenological equations can be used in the estimation of transmembrane permeability coefficients of a selected population of cells; and

5. Finally, in a more general context, to develop a model which can lead to a better understanding of the mechanisms which control movement of solutes during the *osmotic* removal of water from complex cellular structures (here, solutes are understood to be aroma compounds and any soluble solids usually contained within the cells of the original material or which are introduced by the dehydration process).

We hope that the present work may be an incentive for further thinking and involvement in the subject of modeling and simulation of biological systems.

1.2 Outline of the study

The thesis can be divided roughly into two largely self-contained parts: Chapters 2 to 5 consider the *characterization of membrane permeability properties*, and Chapters 7 to 9 present a model for describing *isothermal mass transfer in complex cellular structures*, based in part on theoretical considerations developed in the preceding chapters. For the sake of continuity, we have chosen to keep the two parts relatively independent of one another in the sense that each contains its own literature review, experimental section and discussion. This is to reflect in a way the intrinsic hierarchy which exists when one considers mass transport phenomena in complex cellular structures. One must look first at their basic structural components, the *cells*, before moving to an arrangement of such entities, the *tissue*. Furthermore, the reader will realize that the first part deals more specifically with the practical estimation of membrane permeabilities, whereas the second considers the use of such estimates as part of the information necessary to predict the behavior of whole tissues.

By way of introduction this report starts in Chapter 2 with a review and analysis of the theory of irreversible thermodynamics and its application to the description of transmembrane mass transport phenomena. This outline serves as a general background for Chapter 3 where we propose a novel approach in the characterization of membrane permeability properties in biological systems. Chapter 4 is devoted to discussing the procedures inherent to the numerical estimation of membrane permeability coefficients. The development of the governing equations, an analysis of the nature of the experimental data and a discussion on the statistical analysis of the observations as well as a description of the experimental methods used are covered in detail. Chapter 5 completes this first part of the work by presenting the results of the application of the previous methodology to the characterization of the membrane permeability properties of a selected population of cells.

The preliminaries necessary to introduce the second part of the study are dealt with in Chapter 6. In Chapter 7, the general aspects of the description and modeling of isothermal mass transfer in complex cellular structures are reviewed. This section, as for Chapter 2, serves as a general background for Chapter 8 where we present the details of the development of a new model for the mathematical analysis of mass transfer in such an environment. Chapter 9 presents and discusses the results of simulations carried out to test the model. This section completes the presentation of the second part of the work, which is reviewed in Chapter 10.

Another self-contained section which should be mentioned, in view of its potential use, is Appendix 1. This appendix gives a detailed description of a direct-search optimization algorithm (based on the *Modified Simplex* method of Nelder and Mead [1965a,b]) which has been developed to permit the estimation of the aforementioned membrane properties. This algorithm has proven to be very robust and versatile. The interested reader might therefore find the information very useful in view of possible applications of the algorithm to other problems.

2. CHARACTERIZATION OF MEMBRANE PERMEABILITY PROPERTIES IN BIOLOGICAL SYSTEMS: A REVIEW

2.1 General

Recent years have seen an enormous increase of interest in the function of biological membranes. Arguments based on *membrane properties* are now considered integral parts of biological and medical thinking; one needs only to mention the role ascribed to membrane processes in pharmacology, muscle and nerve physiology or cryobiology. In particular, the study of *membrane transport* is now an important field of research and tremendous efforts are being put into improving the understanding of the governing mechanisms. This, however, cannot be achieved without an *interdisciplinary* approach, especially in view of the fact that many related issues which are biological in nature can be usefully discussed within the same framework as nonbiological problems. Indeed, the availability of a sound theoretical basis in sciences such as chemistry and physics provides the researcher with a foundation of certainty on which to build rigorous analyses of biological systems. The advent of *irreversible thermodynamics* at the beginning of this century and the classical work of Kedem and Katchalsky (1958), who derived from that theory solute and volume flow equations describing mass transport phenomena across biological membranes, are examples of the systematic application of existing science and technology to improve the understanding of biological phenomena. Some aspects of the theory of irreversible thermodynamics and its application to the description of transmembrane mass transport phenomena will be discussed in the remainder of this section.

2.2 Review of existing concepts

2.2.1 Irreversible thermodynamics as a physical theory

Since a complete analysis of the theory of irreversible thermodynamics is not essential in the scope of this presentation, one should refer to these introductory readings for a more detailed treatment: DeGroot and Mazur (1962), Katchalsky and Curran (1967), Chartier, Gross and Spiegler (1975) and Wiśniewski, Staniszewski and Szymanik (1976). Hence, we will concentrate our discussion to showing the practicality of the approach rather than analyzing its theoretical subtleties. The following review mostly parallels that of Lightfoot (1974) and is by no means exhaustive. We will restrict ourselves to presenting the main features of the theory of irreversible thermodynamics (abbreviated *IT* hereafter).

The postulates of the theory of irreversible thermodynamics

A. Entropy production

The quantitative measure of the degree of randomness or disorder in a system is its entropy. From the *Second Law* of classical thermodynamics, any spontaneous change occurring in an *isolated* system is accompanied by an increase in entropy. This tendency towards randomization, i.e. the equalization with the passage of time, makes a spontaneous return to an earlier state unlikely if not impossible. This is the *irreversibility* of a phenomenon.

Diffusion and heat conduction, as well as other natural spontaneous processes, are irreversible and as such are accompanied by an increase in entropy. Therefore, it seems reasonable, if we postulate that the rate of increase of entropy in a system is the potential for any spontaneous irreversible changes, that a complete description of those entropy changes taking place should also provide a description of all spontaneous processes which are possible in the system (Lightfoot, 1974). This assumption is the basis for *IT* analyses.

B. First postulate: local equilibrium

In order to relate the entropy changes to measurable macroscopic thermodynamic quantities such as temperature, pressure, concentration or chemical potentials, we need the first postulate of *IT*, which states that *equilibrium* thermodynamic functions of state can be applied to any elementary volume dV, typical of the system as a whole, provided that departures from local equilibrium are sufficiently small, i.e. that the variation of the state variables or parameters in dV is negligible.

A *local thermodynamic* state is determined, for a substance, at individual points of a system which is not in equilibrium. Elementary volumes of substance surrounding the given points must be so small that the material within them can be treated as homogeneous. At the same time, they should contain a sufficient number of molecules for the principles of statistics and the methods of phenomenological thermodynamics to be applicable to them. The Second Law of thermodynamics is then given a *local formulation* which states that, if coupling phenomena involving an increase in entropy occur at any place, entropy-decreasing phenomena coupled to them may also occur at that same place, but the *overall* effect of all the irreversible phenomena at a given place in space *must always* yield an *increase* in entropy (see Wiśniewski *et al.*, 1976; Schuster, 1983).

The range of validity of the postulate of local thermodynamic equilibrium is determinable phenomenologically *only by experiments* (Miller, 1960). The further the system is from the equilibrium state, the more the assumption of local thermodynamic equilibrium is at variance with reality. As shown by experiments, the first postulate is valid if the gradients of intensive thermodynamic functions, which are decisive in regard to equilibrium in the system under consideration, are small and their local values vary relatively slowly in comparison to the relaxation time of the local state of the substance, that is, the time required for a localized variation in the state of the substance to *equalize* through the differential volume. The first condition corresponds to the requirement that the change in an intensive parameter be small over the molecular mean free path (see Hanley, 1967). The second condition originates from the fact that the entropy concept is introduced in statistical thermodynamics in considering a substance over a period of time that is long compared to the relaxation time of its local state, that is, for a substance in a state of *statistical equilibrium* (Callen, 1960).

Accepting the postulate, we can then use the classical-thermodynamic equations of state and the Gibbs-Duhem relations to explicitly relate the rate of entropy production to a summation of fluxes (thermal energy, matter, momentum) multiplied by their conjugated gradients or forces (see Lightfoot, 1974 and Hanley, 1967 for a complete derivation). However, a nonequilibrium process is described *uniquely* only if the set of consecutive states of the system during realization of the process is known. Therefore, contrary to entropy, the *entropy source strength* and the *dissipation function* (to be introduced later on) are *not state functions* since they depend on the mode of change between given states.

C. Second postulate: linear laws, the phenomenological equations

It is to be expected that more than one irreversible process will take place in most natural systems. It is then obvious that the observed transport phenomena will be caused by the simultaneous interactions of forces of different nature. Thus, any theoretical treatment of the relation between fluxes and forces must include not only the coupling between fluxes and their conjugated forces, but also possible coupling between fluxes and nonconjugated forces.

Generated fluxes, or the effects due to thermodynamic forces, are in the general case functions of all forces present in the system, i.e. for n existing forces:

$$\mathcal{J}_i = f(X_j) \; ; \; i,j = 1,2,\ldots,n \qquad [2.1]$$

where $\mathcal{J}_i$ and X_j are the i-th generalized flux and the j-th generalized differential thermodynamic force, respectively. Although we cannot make any predictions about the qualitative or quantitative dependence of the fluxes on the forces, we can generally assume that this dependence can be described formally by a Taylor or Maclaurin series. Therefore, using a Maclaurin expansion relative to the reference *equilibrium*, we can write for the i-th flux:

$$\begin{aligned} \mathcal{J}_i = {} & \Sigma_j \mathcal{J}_i(X_j=0) + \Sigma_j (\partial \mathcal{J}_i/\partial X_j) X_j + \\ & (1/2!)\, \Sigma_{jk} (\partial^2 \mathcal{J}_i/\partial X_j \partial X_k) X_j X_k + \ldots \\ & i,j,k = 1,2,\ldots, \text{[illegible]} \end{aligned} \qquad [2.2]$$

If the thermodynamic forces become zero, the system advances to an equilibrium state in which the generalized fluxes become zero and, hence:

$$\Sigma_j \mathcal{J}_i(X_j=0) = 0 \; ; \; i,j = 1,2,\ldots,n \qquad [2.3]$$

In the first approximation, that is for states *not very far* from equilibrium, we can confine ourselves to *linear* *relationships* between the fluxes and forces, called *linear phenomenological equations*. The series is then broken off after the second term, leading to:

$$J_i = \Sigma_j L_{ij} X_j \; ; \; i,j = 1,2,\ldots,n \qquad [2.4]$$

This is the second postulate of *irreversible thermodynamics*.

The *proportionality* factors L_{ij}, called *generalized phenomenological coefficients* or *Onsager coefficients* (named after Lars Onsager, who pioneered the development of *IT* [Onsager, 1931]), are thus defined as follows:

$$L_{ij} = (\partial J_i/\partial X_j)|X_k{=}0$$
$$i,j,k = 1,2,\ldots,n \text{ and } j \neq k \qquad [2.5]$$

which is equivalent to:

$$L_{ij} = (J_i/X_j)|X_k{=}0$$
$$i,j,k = 1,2,\ldots n \text{ and } j \neq k \qquad [2.6]$$

The generalized thermodynamic forces and the generalized fluxes are quantities determined, as we will see, by analysis of the expression for the entropy source strength or the dissipation function. The phenomenological coefficients are not functions of the thermodynamic forces, hence, also are not functions of the effects of their action in the form of generalized fluxes; on the other hand, they can be functions of the parameters of the local state of a substance and depend on the kind of substance constituting the system under consideration. The phenomenological theory cannot reveal how small the thermodynamic forces X_j must become so that the linear relationships are a good approximation. This must be checked by experiment (Miller, 1960; Biswal and Le Maguer, 1984) or, for model systems, it can be found from the model equations (Mikulecky, 1978, 1979). However, the second postulate has been shown to be valid for most cases involving fluxes of substance, heat or electric current; the greatest deviations from this postulate appearing for chemical reactions and relaxation phenomena (Miller, 1960).

The system of equations so generated (equation 2.4) describes, therefore, n different fluxes in terms of n different forces. L_{ii} are referred to as *straight-coefficients* signifying the relations between the fluxes and their corresponding conjugate forces X_i. On the other hand, L_{ij} $(i \neq j)$ are called *cross-coefficients*, signifying the dependence of fluxes on *nonconjugated* forces, and prove to be crucial in describing the *interaction phenomena* known to occur in most real systems. The phenomenological coefficients can thus be seen as factors indicating what contribution the force X_j makes to the flux J_i.

As indicated by Wei (1966), this theory is appealing since it recognizes that the movement of one species in a system may affect the flux of a second species. In other words, since it uses the same parameters as classical thermodynamics (through the first postulate), the driving force leading to the flux of any species depends not only on the gradient of its own chemical potential but can be affected by any other force occurring in the system.

[illegible]. Third postulate: Onsager reciprocity relations

The third postulate states that, if for a given system the phenomenological equations are written in terms of n *valid conjugated* fluxes and forces (appearing in the entropy production relation), then the cross-coefficients L_{ij} and L_{ji} ($i \neq j$) are *symmetrical*. The choice of the thermodynamic forces must be made so that in the equilibrium state, when the thermodynamic forces vanish (i.e. $X_j = 0$), the entropy production (dissipation function) must also be zero (for discussion on the restrictions on the third postulate see Wei [1966]). This postulate is particularly important since it reduces the number of coefficients needed to describe a system from n^2 to $.5n(n+1)$.

2.2.2. Application of the theory of irreversible thermodynamics to the description of transmembrane mass transport phenomena

Let us first clarify some concepts related to the use of the theory of irreversible thermodynamics in the study of membrane transport processes and give a clear picture of the nature of the permeability coefficients to be estimated.

As stated by Wei (1966), irreversible thermodynamics can be considered as a *first-level* theory, that is, a theory which is *phenomenological* in nature and which seeks merely to describe a system by *macroscopic* observables and is supposed to be true for *any molecular structure and mechanism*. This theory can therefore give only a *framework* to analyze a system essentially independent of the details of its structure. As soon as we start looking at molecular structures and interaction mechanisms and we try to derive properties from a knowledge, for example, of statistical mechanics or kinetic theory, we start dealing with *second-level* theories and irreversible thermodynamics merges with the more complex field of *statistical thermodynamics* since there is a definite attempt to grasp, at the molecular level, the intrinsic nature of the system. The pioneering work of Kedem and Katchalsky (1961) on the physical interpretation of the phenomenological coefficients of membrane permeability in terms of friction coefficients is an example of such an attempt.

In biological systems transport processes are known to occur in membranes of complex structure. Furthermore, in the study of these transport phenomena, it is almost impossible to have experimental access to events within these membranes or their complex arrangements. The experimenter is therefore restricted to studies which involve variables only in one or both of the bounding phases. Whether the system involves only simple diffusion, more complex diffusion and flow, or whether transport is coupled with actual chemical reactions as may be involved in active or facilitated transport, the analysis of the problem by means of irreversible thermodynamics offers some distinct advantages since it requires no mechanistic details. However, it yields little information about the membrane system as could be expected from a second-level theory. We have to realize that a *macroscopic* approach is most appropriate not because of a lack of theoretical tools but because of a lack of *microscopic* information. The question therefore is: "How can we use the concepts of irreversible thermodynamics to derive dynamic relations describing our system?", or, more importantly: "What is the nature of the information that we can expect from such treatment?".

The nature of the phenomenological coefficients

When dealing with the *macroscopic* description of irreversible processes across biological membranes, there are generally two accepted approaches: (1)the *integration* approach, which consists in integrating the *local* equations describing the entropy production *within* the membrane over the region of the barrier (DeGroot and Mazur, 1962; Katchalsky and Curran, 1967; Kirkwood, 1954), and (2), the "*black-box*" approach, which considers the entropy production for the *entire* system, that is, the two phases separated by the transporting layer *together with* the possible existence of unstirred layers adjacent to the physical structure (Sauer, 1973).

Although the final *global* phenomenological equations derived from both treatments are essentially of the same form, a close look at the conditions that must be fulfilled in the former approach to ensure that the Onsager reciprocity relations are valid, shows that this method is far more restricted in terms of its domain of application than the latter one (Snell and Stein, 1966). In fact, as discussed by Mikulecky (1978, 1979), it can be shown that the resultant integrated global equations do not obey Onsager reciprocity for cases where the *local* equations of motion take into account the viscous dissipative forces and the position dependence of the local friction coefficients. Mickulecky therefore questions the soundness of the local description approach to simulate a macroscopic behavior, especially in view of the fact that the global phenomenological coefficients are very complicated functions of local friction and viscosity coefficients and, consequently, doubts our ability to get information about molecular events from macroscopic transport measurements.

The "*black-box*" approach seems more appropriate since it is truly phenomenological in nature. The question remains whether or not reciprocal relations can be expected to hold for the overall coefficients under this treatment. In his work, Sauer (1973) shows that, under very general conditions, the black-box approach termed *nonequilibrium thermodynamics of discontinuous systems* preserves the reciprocity relations. This rather paradoxical result is nevertheless perfectly logical. Indeed, it only confirms that, in all systems where a level-two understanding is not available, the Onsager reciprocity relations hold regardless of our lack of understanding of molecular structure and mechanism, provided, however, that the problem is kept within the framework of the theory. Furthermore, it reveals that there are no simple relationships between the global phenomenological coefficients generated by both methods, that is, that the discontinuous treatment yields "*effective*" or "*apparent*" coefficients that cannot be compared to integrated parameters which are strongly dependent on the accuracy of the microscopic analysis of the system. To paraphrase Mikulecky (1979), we can see the latter point as proof that the whole system is more than a mere sum of its parts.

2.2.3 Nonequilibrium thermodynamics of discontinuous systems

Transport of components without chemical reactions

Taking the approach of nonequilibrium thermodynamics of discontinuous systems, the entropy production in a biological membrane can be obtained as follows. Referring to Figure 2.1, let us define a single cell and its suspending medium as a *closed* thermodynamic system of nonspecified shape composed of two homogeneous liquid phases 1 and 2 separated by a membrane in *steady-state*, that is, its thermodynamic state is time invariant. The steady-state condition implies that the barrier does not contribute to the variation of the system entropy since there is neither matter nor energy accumulation allowed in it. The membrane is assumed to be merely a *passive* diathermal wall which controls, by its selective properties, the dynamic of the

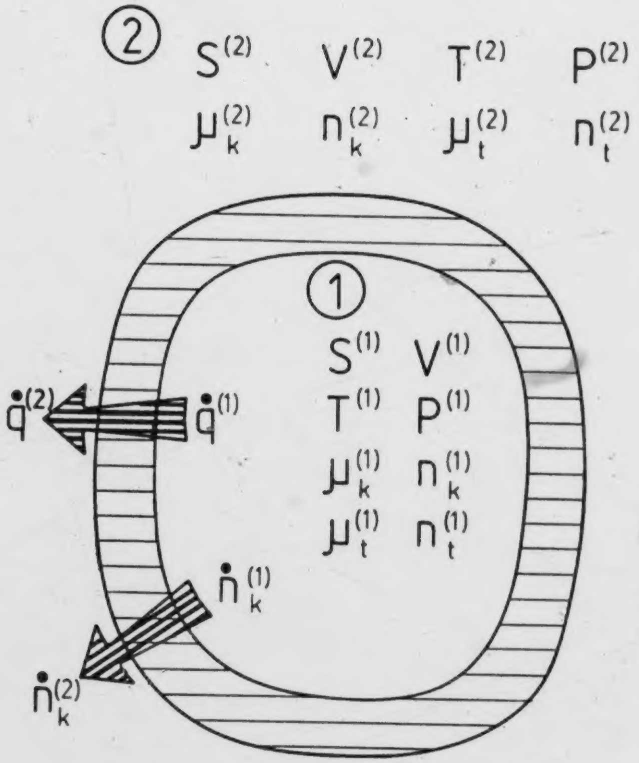
②
$S^{(2)}$
$V^{(2)}$
$T^{(2)}$
$P^{(2)}$
$\mu_k^{(2)}$
$n_k^{(2)}$
$\mu_t^{(2)}$
$n_t^{(2)}$
①
$S^{(1)}$
$V^{(1)}$
$T^{(1)}$
$P^{(1)}$
$\mu_k^{(1)}$
$n_k^{(1)}$
$\mu_t^{(1)}$
$n_t^{(1)}$
$\dot{q}^{(2)}$
$\dot{q}^{(1)}$
$\dot{n}_k^{(1)}$
$\dot{n}_k^{(2)}$

Figure 2.1 Membrane in a steady-state incorporated into a closed thermodynamic system.

exchanges between phases 1 and 2. In this case it is defined as the *total region of inhomogeneity*, including, for example, *unstirred layers* (see Sauer, 1973). It is important for the following analysis to realize that we will not attempt to differentiate the "biological membrane" from adjacent unstirred layers. We therefore assume that the boundary effects are lumped into the membrane properties.

Each of the homogeneous phases is a multicomponent liquid phase consisting of m species, either permeable (subscript k, k = 1,2,...,n) and/or impermeable (subscript t; t = [n+1],[n+2],...,m), and each is defined by a set of intensive and extensive quantities, namely, S the entropy of the compartment, V the volume, T the absolute temperature, P the pressure, μ_k (μ_t) and n_k (n_t), the chemical potential and the number of moles of the k-th (t-th) component, respectively.

Since it is reasonable to assume that one of the mechanisms influencing the flow-force relations (to be discussed below) involves interactions of the diffusing species with the membrane, we must include the membrane as one of the species present in the closed system. Furthermore, the membrane should be considered as a multicomponent single *nonuniform* phase (see Hanley, 1967). For convenience, nevertheless, we will restrict the use of the terms "*phase*" or "*bath*" to describe the two solution compartments.

The two compartments are allowed to communicate thermally by an energy flow $\dot{q}$, mechanically by configurational alterations and chemically by transmembrane flows of the solution *permeable* species $\dot{n}_k$. The composition of each phase is assumed to be uniform but not necessarily the same as the composition of the other phase. The postulate of local thermodynamic equilibrium is, in this case, replaced by the requirement that the variations in the intensive and extensive parameters be slow enough for each of the phases to be in thermodynamic equilibrium at every instant. The thermodynamic quantities of each phase are only functions of time, whereas at the interface they may change by jumps (see Wiśniewski *et al.*, 1976). This requirement is equivalent to assuming that all departures from local equilibrium occur at the *interfaces* of the region of inhomogeneity.

Since these two phases as well as the membrane are thermodynamic -*open* systems, the process considered results from a change from a nonequilibrium state toward an equilibrium state with respect to the distribution of species among the phases. It is therefore to be expected that this nonequilibrium situation will be characterized by flows $\dot{n}_k$ defined by the *rate of appearance* (or disappearance) of the k-th species on the two sides of the membrane, that is:

$$\dot{n}_k = dn_k/d\theta \qquad [2.7]$$

where θ is the time variable. Let us recall that it is assumed that there are no chemical reactions occurring in the system.

The entropy production (entropy source strength) in the membrane can then be written in terms of only those quantities observable in the media on the two sides of the barrier through the use of the Gibbs equation for open thermodynamic systems. This way of looking at a discontinuous system is thus to look at the system as a *whole*. No information is available on the irreversible processes occurring *inside* the membrane, only the *effects* of these processes on phases 1 and 2 are noted (Staverman, 1952).

It can be shown, and the reader is referred to the treatment of Sauer (1973) for the complete derivation, that the *internal* change of entropy of the system due to the irreversible processes in the membrane, $dSi/d\theta$, is given by:

$$dSi/d\theta = \dot{\underline{q}}^{(2)}\Delta(1/T) + \Sigma_k \dot{n}_k^{(1)}\Delta(\mu_k/T) + \Sigma_t \dot{n}_t^{(1)}\Delta(\mu_t/T) \geq 0$$

$$k = 1,2,\ldots,n \text{ and } t = n+1,n+2,\ldots,m \qquad [2.8]$$

where the summations are taken over the n permeable species and the (m-n) impermeable ones, respectively, and Δ is defined as the difference between quantities 2 and 1, that is, $\Delta = (2) - (1)$. Note that the following sign convention is also adopted, namely: flows (fluxes) *going out* of the cellular volume (phase 1) are *negative*, i.e. equivalent to a *loss*, whereas flows (fluxes) *going into* phase 1 are *positive*, i.e. equivalent of a *gain*.

With the steady-state assumption for the membrane region, i.e. from the energy and matter standpoint the membrane is only a jump condition, the flow of energy is constant through the barrier, namely:

$$\dot{q}^{(1)} + \dot{q}^{(2)} = 0 \qquad [2.9]$$

The same is true for the flow of the k-th *permeable* species since no chemical reactions occur within the membrane, namely:

$$\dot{n}_k^{(1)} + \dot{n}_k^{(2)} = 0 \qquad [2.10]$$

The above statements mean that any energy and/or matter appearance on one side of the barrier is the result of the disappearance (transfer) of an equivalent quantity on the other side. In the case of *nonpermeable* components, the flow of the t-th species is simply null on both sides since $\dot{n}_t$ = 0 in both compartments. Equation 2.8 can therefore be rewritten as:

$$dSi/d\theta = \dot{q}^{(2)}\Delta(1/T) + \Sigma_k \dot{n}_k^{(1)}\Delta(\mu_k/T) \geq 0$$
$$k = 1,2,\ldots,n \qquad [2.11]$$

Note that the last term of equation 2.8 does not appear in equation 2.11 because the flow of the t-th species is null and not because the gradient of chemical potential for that species vanishes, i.e., $\Delta(\mu_t/T) = 0$.

In the special case of an *isothermal system*, equation 2.11 takes a form which is often referred to as the *dissipation function*, Ψ (Katchalsky and Curran, 1967), namely:

$$T\, dS_i/d\theta \equiv \Psi = \Sigma_k \dot{n}_k^{(1)} \Delta\mu_k \geq 0 \qquad k = 1,2,\ldots,n \qquad [2.12]$$

Chemical reactions have been deliberately omitted in the preceding analysis because progression rates as well as reaction driving forces are seldom accessible to direct evaluation. The interested reader may refer to the work of Sauer (1973), Spangler (1977) or Frömter (1983) for more details.

Restricting further consideration to the isothermal case, we can now use the concepts of irreversible thermodynamics to derive *macroscopic phenomenological relations*, written analogously to equation 2.4, of the form:

$$J_j^{(1)} = \Sigma_k L_{jk} \Delta\mu_k \; ; \; j,k = 1,2,\ldots,n \qquad [2.13]$$

where L_{jk} is the jk-th *macroscopic phenomenological coefficient* (Onsager form for isothermal systems) relating the *flux* of matter $J_j^{(1)}$ to the driving force $\Delta\mu_k$ and obeying the Onsager reciprocity relation $L_{jk} = L_{kj}$, $j \neq k$. We have expressed here the rate of appearance or disappearance in bath 1 of the j-th permeable component per *unit surface area* of the membrane, that is:

$$J_j^{(1)} = (1/A)\, dn_j^{(1)}/d\theta \qquad [2.14]$$

where A is the membrane surface area.

It must be emphasized here that a vanishing flux, i.e. $J_j^{(1)} = 0$, may be attributable either to the entire set of macroscopic phenomenological coefficients (straight- and cross-coefficients) being zero, although gradients in chemical potentials might exist, i.e.: $L_{jk} = 0$ and $\Delta\mu_k \neq 0$; $j,k = 1,2,\ldots n$ (this is the case of the *impermeable* components), or to the absence of a thermodynamic force for the j-th species coupled with null cross-coefficients, i.e. $\Delta\mu_j = 0$ and $L_{jk} = 0$; $j,k = 1,2,\ldots,n$ and $j \neq k$.

Independence of fluxes and forces

The isothermal entropy production (equation 2.12) and the related phenomenological equations (equation 2.13) are written implicity in terms of the fluxes of the m species composing the homogeneous phases and their conjugate forces. In this treatment of membrane permeation as a discontinuous system, the membrane structure itself plays the role of the (m+1)-th component. However, being confined to the open thermodynamic region of inhomogeneity, this species does not appear explicitly as part of the entropy production relation since there is no possible redistribution of the component "*membrane*" between the two solution phases, in other words, the membrane does not contribute to the nonequilibrium state of the closed system, i.e. $\Delta\mu$(membrane) and $\dot{n}$(membrane) are null.

An inspection of the physical arrangement of our system shows that there are no concentration gradients in phases 1 and 2, all the gradients in chemical potential being found within the region of inhomogeneity where no experimental study of the forces is possible. The difficulty with the use of the *differences* in chemical potential, $\Delta\mu_k$ $(\Delta\mu_t)$, is that these quantities represent a difference between the chemical potentials of the k-th (t-th) component *in* the membrane *at the boundaries*; these values of μ's are unknown and not available to experimental determination. However, following Kirkwood (1954), we will make the assumption that the chemical potentials *at the surface* are the same as the corresponding potentials *in the adjacent solutions*. Therefore, since the membrane species is absent from both phases, its chemical potential in the solutions is zero, which implies that, for the region of inhomogeneity, $\Delta\mu$(membrane) is null (see Katchalsky and Curran, 1967).

Yet, it is important to include the membrane as a component since it affects the number of *independent* fluxes and forces in the system. For instance, in the foregoing analysis, we have (m+1) components distributed between 3 phases. In each phase we can state the composition by taking one component as a point of reference and specifying the amount of each of the other components relative to the unit quantity of the reference component. In any case, we then have m composition variables for each phase; hence, there are 3m such variables for all 3 phases, i.e. we can write for each phase m *independent* rates of appearance (or disappearance), some of which may be eliminated by writing *supplementary* relations.

Turning now to the driving forces, we have, as well, (m+1) gradients related to the (m+1) rates. By making use of the integrated Gibbs-Duhem equation (for *isothermal* and *isobaric* conditions; see Sauer, 1973 and Frömter, 1983 for details), namely:

$$\Sigma_j \bar{c}_j \Delta\mu_j \cong 0 \; ; \; j = 1,2,\ldots,m+1 \qquad [2.15]$$

where $\bar{c}_j$ is the average concentration of species j *across* the membrane, we can therefore express one of the driving forces as a function of the others. Hence, we have m *independent* rates and m *independent* driving forces of which, again, some may be eliminated by introducing supplementary relations.

Let us take, for example, a system composed of 2 ternary solutions, say, 2 permeable solutes (x and y) in a solvent (s), separated by a membrane (m). It follows from the preceding discussion that the transport phenomena occurring in that system can be completely described by the following flows and forces. For each bath, we can write the following rates: $\dot{n}_s$, $\dot{n}_x$, $\dot{n}_y$ and $\dot{n}_m$, of which only 3 are independent. For instance, if we select the solvent as reference, we can write each rate relative to the solvent, leading to: $\dot{n}'_x = d(n_x/n_s)/d\theta$, $\dot{n}'_y = d(n_y/n_s)/d\theta$ and $\dot{n}'_m = d(n_m/n_s)/d\theta$. The latter can be eliminated using the supplementary relation $n_m = 0$ in both baths. Corresponding to these flows are 3 independent forces, since (from equation 2.15):

$$\bar{c}_s\Delta\mu_s + \bar{c}_x\Delta\mu_x + \bar{c}_y\Delta\mu_y + \bar{c}_m\Delta\mu_m = 0 \qquad [2.16]$$

We have, nevertheless, the supplementary relation $\Delta\mu_m = 0$. Therefore, to the 2 remaining independent rates, $\dot{n}'_x$ and $\dot{n}'_y$, correspond the following independent difference gradients $\Delta\mu_x$ and $\Delta\mu_y$.

On the other hand, if one prefers to stay with the flows expressed as: $\dot{n}_s$, $\dot{n}_x$, $\dot{n}_y$ and $\dot{n}_m$, the supplementary relation $n_m^{(1,2)} = 0$ can be used to eliminate $\dot{n}_m$, leaving the 3 independent rates: $\dot{n}_s$, $\dot{n}_x$ and $\dot{n}_y$, to which the forces $\Delta\mu_s$, $\Delta\mu_x$ and $\Delta\mu_y$ are conjugated. Note, nevertheless, that, due to the Gibbs-Duhem relation (equation 2.16) and the supplementary relation $\Delta\mu_m = 0$, the solvent driving force becomes a function of the gradients of the 2 solutes, that is:

$$\Delta\mu_s = f(\Delta\mu_x, \Delta\mu_y) \qquad [2.17]$$

The choice of a frame of reference

Since fluxes theoretically can (but need not) be related to velocities, the question of the choice of a frame of reference for expressing those rates has been raised, particularly in the case of cell shrinkage observations (Meares, 1977). Although this question is crucial in the case of the integration approach, where the study is made on a volume element within the membrane (Mikulecky and Caplan, 1966), it is, in the discontinuous analysis, completely immaterial since, as far as the entropy production is concerned, the membrane, moving or not, is a black-box separating two baths, the properties of which might change with time. In fact, as stated by Spangler (1977), the fluxes can be expressed with respect to the velocity of the membrane, leaving m independent fluxes. The question of the moving frame of reference in cell shrinkage observations does not however pose a problem since the nature of the observations (which corresponds, as we will see, to observing what happens in one or both baths), is equivalent to "*sitting*" on the membrane while monitoring the rates of appearance or disappearance on both sides. In that case, the frame of reference, moving or not, does not affect the observations.

We have to realize that it is mostly through the observation of the macroscopic effects of those changes in one or both media and through some indirect simulations, that estimates of the membrane phenomenological coefficients, commonly termed *permeability coefficients*, will be made and not by direct measurements at the microscopic level. It is then evident that these coefficients are "*effective*" or "*apparent*" *macroscopic coefficients* and must be considered as such. This should become clear as we progress in the present analysis and introduce supplementary assumptions to facilitate the determination of these coefficients.

2.2.4 The operational form of the phenomenological relations reexamined

In their original treatment, Kedem and Katchalsky (1958) introduced a form of the phenomenological relations referred to as the "*practical*" flux equations, based on forces usually employed in permeability studies, namely:

$$J\upsilon = Lp(\Delta P - \Sigma_t \Delta\pi_t - \Sigma_k \sigma_k \Delta\pi_k)$$
$$k = 2,3,\ldots,n \text{ and}$$
$$t = n+1,n+2,\ldots,m \qquad [2.18]$$

where $J\upsilon$ is the volume flux, defined as:

$$J\upsilon = \Sigma_k \tilde{v}_k J_k \;;\; k = 1,2,\ldots,n \qquad [2.19]$$

where $\tilde{v}_k$ is the partial molar volume of species k. Lp is the *membrane permeability to water*, often referred to as *hydraulic conductivity* (*permeability*) or *filtration coefficient* and σ_k is the *reflection coefficient* of species k. For obvious reasons, they introduced a notation familiar to the membrane scientist, who thinks of driving forces across a membrane not in terms of difference in chemical potential $\Delta\mu_i$, but rather in terms of the hydrostatic pressure difference ΔP, the electromotive force E, and the species concentration differences given either as difference of concentration ΔC_i or in pressure units as $\Delta\pi_i$. Note that we have used here the notation introduced previously to designate the permeable and impermeable species present in the system. We have also selected species 1 to represent the solution *solvent* which, in the case of biological studies, is obviously *water*.

Concomitantly, the molar flux of the k-th permeable solute is given (Biber and Curran, 1968) by:

$$J_k = \bar{C}_k(1-\sigma_k)J_v + \omega_{kk}\Delta\pi_k + \Sigma_j\omega_{kj}\Delta\pi_j$$
$$j,k = 2,3,\ldots,n \text{ and } j \neq k \qquad [2.20]$$

where, as before, $\bar{C}_k$ is the average concentration of species k across the membrane, ω_{kk} is the k-th *solute permeability coefficient* and ω_{kj} is a *generalized permeability coefficient*. Note that the latter coefficients are *not symmetrical*, i.e. $\omega_{kj} \neq \omega_{jk}$. They are, nevertheless, related through a *linkage coefficient* $\lambda_{(kj)}$ (Hoshiko and Lindley, 1967) as follows:

$$\omega_{kj}\bar{C}_j = \omega_{jk}\bar{C}_k = \lambda_{(kj)} \qquad [2.21]$$

The filtration coefficient *Lp* is, by definition, a measure of the filtering capacity of the membrane when a pressure difference ΔP is set up between the two sides of the barrier. This coefficient expresses the resistance to volume flow as if the dynamic of fluid flow was controlled by *Poiseuille's Law*, i.e. as if the volume flow was occurring in cylindrical pores. Since, in reality, it is not certain that pores exist in biological membranes, this coefficient is an *apparent* hydraulic permeability and the *microscopic* interpretation of *Lp* is not a simple matter.

The same is also true for the solute permeability coefficient, ω_{kk}, which measures the resistance to mass transfer opposed by the membrane as if the dynamic of the solute flow was controlled by *Fick's Law*. It is analogous to an *apparent mass transfer coefficient* characterizing the flow of the solute generated by a gradient of its concentration across the membrane. The generalized permeability coefficients, on the other hand, describe the effects of solute-solute interactions in the membrane as the species diffuse through the barrier.

The reflection coefficient, σ_k, is related, for its part, to the concept of *pore hindrance factor* (Villars and Benedek, 1974). It represents the fraction of solute molecules "*rejected*" by the pseudopores due to the ratio molecule-pore size and due to an additional drag on the diffusing molecules caused by the proximity of the pore walls.

It must be emphasized, however, that, whereas these parameters, when estimated for biological membranes, can be related to "*equivalent*" pore models, it is by no means a proof that such membranes contain pores. In fact, the terminology has been extended to biological studies as a result of the attempt, by membrane scientists, to explain biological membrane behavior from investigations into artificial membranes.

Yet, in spite of being "*practically*" appealing, there has been some concern that these equations were lacking generality and some authors proposed modified practical forms to take into account, for example, nonlinear transport behavior (Schlögl, 1969; Richardson, Foster and Miekisz, 1982) or transport in the presence of nonideal, nondilute bathing solutions (Friedman and Meyer, 1981). Futhermore, it can be shown that the "practical" parameters are complex functions of the macroscopic phenomenological coefficients present in equation 2.13.

For example, let us consider a system composed of a membrane separating 2 binary solutions composed of a permeable nonelectrolyte (x) and a solvent (s). The hydraulic permeability has the following value (Nobel, 1983):

$$Lp = \tilde{v}_s^2 L_{ss} + 2\tilde{v}_x \tilde{v}_s L_{sx} + \tilde{v}_x^2 L_{xx} \qquad [2.22]$$

whereas the reflection coefficient is given by:

$$\sigma_x = [\tilde{v}_s^2 L_{ss} - (\tilde{v}_s/\bar{c}_x)L_{sx} - (\tilde{v}_x/\bar{c}_x)L_{xx} + \tilde{v}_x \tilde{v}_s L_{sx}]/Lp \qquad [2.23]$$

and the solute permeability by:

$$\omega_x - [\bar{c}_x(1-\sigma_x)\sigma_x Lp] = (L_{xx}/\bar{c}_x) - L_{xs}\tilde{v}_s \qquad [2.24]$$

As the number of permeable solutes increases, these relations become intractable unless some simplifying assumptions are made (see Chartier *et al.*, 1975), therefore, we will not attempt to generalize them. However, the above example is sufficient to illustrate the *intrinsic interdependence* of the practical permeability coefficients and the potential violation of the basic statistical requirement of parameter independence if one wishes to estimate their values simultaneously from a common experiment. We have to remember that, traditionally, *independent* experiments were carried out to estimate these parameters and, although the approach is still employed in artificial membrane studies, the evolution in the techniques used in the biological field makes this approach unnecessary.

We feel, therefore, that the analysis of transport through biological membranes could equally well be described by the more *fundamental* form of the phenomenological equations (equation 2.13) as proposed in fact by Staverman (1952) or Kirkwood (1954) before Kedem and Katchalsky's analysis (1958). The main advantage of this form is that it not only preserves the true independence of the coefficients but it also implicity allows extension to nonideal solutions as well as taking into account any electrical interaction between permeable ions through the intrinsic definition of the gradient in chemical potential used as driving force, that is:

$$\Delta\mu_k = \mathcal{R}T\Delta\ln a_k + \tilde{v}_k\Delta P + z_k F\Delta\Phi \qquad [2.25]$$

where $\mathcal{R}$ is the universal gas constant, T is the absolute temperature of the system, $\Delta\ln a_k$ the gradient of molecular potentials, ΔP the hydrostatic pressure difference between both baths and $\Delta\Phi$ the electrical potential difference. $\tilde{v}_k$

and z_k are, respectively, the partial molar volume and the electrical charge (ionic valency) of species k; F is the Faraday constant.

Another point in favor of the suggested approach resides in the *properties* of the macroscopic phenomenological coefficients. Indeed, as we have seen previously, they are, firstly, symmetrical, which proves useful since it reduces the number of independent coefficients. Secondly, based on theoretical considerations (see Wiśniewski *et al.*, 1976), they are constrained within a certain range of possible values obeying the following limits, namely:

$$L_{kk} > 0 \qquad [2.26]$$

and

$$|L_{jk}| \leq \sqrt{(L_{jj}L_{kk})} \qquad [2.27]$$

Let us note that, whereas the straight-coefficients must always be larger than zero, the cross-coefficients can be either positive, which indicates *mutual drag* between the fluxes J_j and J_k, or negative, which indicates that the transport of species j in one direction creates a flux of species k in the *opposite* direction (Kedem and Katchalsky, 1963).

The following section will be devoted to presenting how the definition of the macroscopic phenomenological coefficients is extended in view of their use in the characterization of permeability properties of biological membranes based on the application of the proposed fundamental form of the phenomenological relations (equation 2.13).

3. A MORE RIGOROUS APPROACH IN THE DEFINITION OF MEMBRANE MACROSCOPIC PERMEABILITY COEFFICIENTS

As stated by Frömter (1983), the phenomenological coefficients, which describe the transport behavior of the system, depend not only on the properties of the region of inhomogeneity but also on the intensive state functions of the entire system. As we have seen in section 2.2.1, these coefficients are, by virtue of the postulates of *IT* and the ensuing theoretical derivations, the *partial derivatives* of the fluxes with respect to the forces evaluated *at equilibrium*, that is, when all thermodynamic forces but one vanish, which, applied to our system, is given by:

$$L_{ij} = (\partial J_i/\partial \Delta\mu_j)|\Delta\mu_x{=}0$$

$$i,j,x = 1,2,\ldots,m \text{ and } j \neq x \qquad [3.1]$$

Practically speaking, as long as the validity of the phenomenological relations is ensured, that is, the system under consideration is within the linear region, the same result should be found if all forces except $\Delta\mu_j$ are held *constant* during the measurement.

The above relation is important in two ways. Firstly, it defines the conditions to be fulfilled to allow the evaluation of the coefficients and, secondly, it shows the *independence* of the coefficients from the thermodynamic forces involved in the system. On the other hand, it does not rule out relationships of the coefficients with the intensive and extensive properties of the bathing solutions, as has been observed, for instance, by Rich and coworkers (Rich, Sha'afi, Romualdez and Solomon , 1968) in their study of the effect of osmolality on the hydraulic permeability coefficient of the red cells. Furthermore, equation 3.1 reveals that, for all species that cannot cross the region of inhomogeneity, their related coefficients are explicitly set to 0, although a gradient in their chemical potential might exist.

From the above discussion it is clear that, if one considers using these estimates for prediction purposes, the macroscopic phenomenological coefficients should be expressed in a form which makes them relatively independent of the properties of the system. In the study of biological systems, it is reasonable to expect the coefficients to be composition and temperature dependent (the pressure dependence can be neglected in the case of animal cells [Rand, 1964b] but not in the case of plant cells [Nobel, 1983]). Since these dependences cannot be derived *a priori* and can be very complex, one has to rely on approximations to characterize these relationships.

As elaborated by Schlögl (1969), if we imagine the fluid crossing the barrier as a relatively dilute solution of permeable species in water, we can expect, as a first approximation, the following composition dependences (expressed as concentrations):

$$L_{kk} \simeq C_k^o \; ; \; k = 1,2,\ldots,n \qquad [3.2]$$

and

$$L_{jk} \approx \sqrt{(C^{\circ}_{j}\ C^{\circ}_{k})}\ ;\ j,k = 1,2,\ldots,n \text{ and } j \neq k \qquad [3.3]$$

where C°_{j} and C°_{k} are *reference* concentrations for the j-th and k-th permeable species, respectively. The choice of the reference concentrations is arbitrary. They are usually selected to fit the experimental conditions (Frömter, 1983; Sauer, 1978). For convenience, we will adopt *symmetric* references in the form of the *arithmetic averages* of the concentrations of the j-th and k-th species in both phases, that is:

$$C^{\circ}_{j(k)} \equiv \bar{C}_{j(k)} = .5\ (C^{(1)}_{j(k)} + C^{(2)}_{j(k)}) \qquad [3.4]$$

The first proportionality (equation 3.2) can be seen as a measure of the apparent permeability of species k, þ_{kk}, which can be defined as:

$$\text{þ}_{kk} = L_{kk} \ /\ \bar{C}_{k} \qquad [3.5]$$

The second relation (equation 3.3) describes the interaction between species j and k and is assumed to be proportional to the probability of an encounter of both species while traversing the region of inhomogeneity. Expressed in a similar fashion, þ_{jk} is defined as:

$$\text{þ}_{jk} = L_{jk} \ /\ \sqrt{(\bar{C}_{j}\ \bar{C}_{k})} \qquad [3.6]$$

The geometric average of the mean concentrations is a necessary outcome of equation 3.5.

The temperature dependence can be taken into account by use of the *Arrhenius* equation (Hempling, 1967; Diller and Bradley, 1984) so that the phenomenological coefficients are explicitly made temperature independent through the following relations:

$$L_{kk} = L^{\circ}_{kk} \exp[(A_{kk}/\mathcal{R})(1/T^{\circ} - 1/T)] \qquad [3.7]$$

and

$$L_{jk} = L^{\circ}_{jk} \exp[(A_{jk}/\mathcal{R})(1/T^{\circ} - 1/T)] \qquad [3.8]$$

where L°_{kk} and L°_{jk} are the values of the phenomenological coefficients at the *reference* absolute temperature, T° (the choice of that reference temperature is also arbitrary), and A_{kk} and A_{jk} are the *apparent transfer energies*, often referred to as *heats of activation* or *activation energies*. $\mathcal{R}$ is, as before, the universal gas constant and T, the absolute temperature of the system under study.

If we now suppose that the constants L°_{kk} and L°_{jk} obey the concentration dependences described above, the macroscopic phenomenological coefficients can be rewritten in terms of parameters showing *minimum* relationship with the properties of the system, namely:

$$L_{kk} = \text{Þ}^{\circ}_{kk}\, \bar{C}_{k} \exp[(A_{kk}/\mathcal{R})(1/T^{\circ} - 1/T)] \qquad [3.9]$$

and

$$L_{jk} = \text{Þ}^{\circ}_{jk}\, \sqrt{(\bar{C}_{j}\, \bar{C}_{k})} \exp[(A_{jk}/\mathcal{R})(1/T^{\circ} - 1/T)] \qquad [3.10]$$

Note that the term "*macroscopic permeability coefficients*" will hereafter refer to $Þ_{kk}$ and $Þ_{jk}$. The superscript ° indicates the form which is temperature independent, i.e. the values of the coefficients at the reference temperature, T°.

As can be seen, the introduction of the Arrhenius relation in the description of the phenomenological coefficients *doubles* the number of estimates. There are, nevertheless, statistical limitations imposed on such analysis (to be discussed in section 4.3.2).

It still remains to be determined if the symmetrical property applies to the macroscopic permeability coefficients, their temperature-independent forms and the apparent transfer energies. If we consider that the apparent transfer energy, A_{jk}, is a measure of the free enthalpy rise necessary for the pair j-k to cross the barrier, it is reasonable to assume the symmetry of that coefficient since it can be envisaged more as a property of the jk-th *couple* than of each individual species versus the other. It then follows that, since the Onsager coefficients are symmetrical by definition and that the use of relation 3.6 does not affect this symmetry, the coefficients $Þ^{\circ}_{jk}$ and $Þ^{\circ}_{kj}$ $(j \neq k)$ are, as well, symmetrical.

4. EXPERIMENTAL: NUMERICAL ESTIMATION OF MEMBRANE MACROSCOPIC PERMEABILITY COEFFICIENTS

If we accept the phenomenological relations (described by equation 2.13) as a valid macroscopic description of the mass transport phenomena occurring across biological membranes (assuming *passive* transport only), it is possible through this dynamic model to simulate the behavior of a single cell put into contact with osmotic solutions, provided that the macroscopic permeability coefficients of the studied cell are known. Conversely, the same equations could be used to estimate these coefficients by using proper statistical techniques to fit the observed cell behavior to the theoretical model, a process referred to as *osmometric study* by House (1974). In this method, differential equations, derived from basic volume and molar balances, are used, in conjunction with appropriate integration routines and fitting procedures, to estimate the phenomenological coefficients as proposed by Stusnick and Hurst more than a decade ago (Stusnick and Hurst, 1972).

This chapter will be devoted to showing how the previous theoretical derivations can be used in the context of osmometric studies to estimate cellular macroscopic permeability coefficients and their related parameters.

4.1 Application of the fundamental phenomenological relations to osmometric studies

4.1.1 The governing equations

Referring to Figure 2.1, the governing equations which model the *macroscopic* behavior of the biological system under investigation are developed as follows.

Considering a single cell immersed in an osmoticum, we can relate the change in *volume* of compartment 1 to the transmembrane fluxes of the *permeable* species, through a relation of the form:

$$dV^{(1)}/d\theta = A\ \Sigma_k \tilde{v}_k J_k^{(1)}\ ;\ k = 1,2,\ldots,n \qquad [4.1]$$

where $V^{(1)}$ is the volume of compartment 1, that is, the *cellular volume*, A is the surface area of the cell membrane, $\tilde{v}_k$ and $J_k^{(1)}$ are, as before, the partial molar volume and the flux (relative to the cellular volume) of the k-th *permeable* species, respectively. Equation 4.1 supposes that the variation in cellular volume is related solely to the volume lost or gained by the compartment due to transmembrane exchanges. It assumes that the partial molar volumes of the transferred species are constant and does not consider volumetric rearrangements that could occur in response to increased (decreased) molecular interactions between the intracellular components.

The change in the *intracellular concentrations* (permeable *and* impermeable species) is written analogously, leading to, for the j-th species:

$$dc_j^{(1)}/d\theta = (A/V^{(1)})\, J_j^{(1)} - (c_j^{(1)}/V^{(1)})\, dV^{(1)}/d\theta$$

$$j = 2,3,\dots,m \qquad [4.2]$$

where the first term describes the contribution of the transmembrane flux of species j to the overall change, whereas the second term describes the effect of the compartment volume change on the concentration variation. Note that for an *impermeable* species the first term vanishes since there is no transmembrane flux for that compartment.

As can be seen, equations 4.1 and 4.2, along with the supplementary relation 2.13, form a system of *ordinary differential equations* which can be solved, using proper techniques, to simulate the volume and concentration variations of the depicted cell assuming, though, that this model is a sufficient description of the transient behavior of the system.

One necessary outcome of the use of relation 2.13 to predict the flux of the j-th species is the necessity to follow the change of the component chemical potential. This is done through equation 2.25 applied as a *point* function in time. Hence, restricting the discussion to the mass transport of *nonelectrolytes* and neglecting the gradient of hydrostatic pressure[1], equa~~tion 2.25~~ simplifies to:

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[1] As demonstrated by Rand (1964a,b), the plasma membrane does not possess the inherent resistance to induce a significant build-up in intracellular hydrostatic pressure in response to inflow of material. Animal cells and plant cells (the cell walls of which have been removed) are therefore prone to burst when put in *hypotonic* bathing solutions.

$$\Delta\mu_j = \mathcal{R}T\Delta\ln a_j \ ; \ j = 1,2,\ldots,m \qquad [4.3]$$

The utilization of the above relation is nevertheless difficult since the access to the value of the *activity* of the j-th species, a_j, is almost impossible in complex multicomponent systems. We have therefore to rely on *approximations* to estimate the chemical potential of the components. These approximations are based on the assumption that the bathing solutions are *ideal dilute phases* which remain so during osmotic transients. Thus, referring to Nobel's treatment (Nobel, 1983) and selecting component 1 to represent the solvent in our system, the difference in chemical potential of water is given by:

$$\Delta\mu_1 \cong -\mathcal{R}T\ \tilde{v}_1\ \Sigma_j(c_j^{(2)} - c_j^{(1)}) \qquad j = 2,3,\ldots,m \qquad [4.4]$$

Equation 4.4 is derived from the *fundamental osmotic pressure law* as applied to *ideal dilute* solutions, leading to a form usually referred to as the *conventional* osmotic pressure law or the *Van't Hoff* relation, that is:

$$\mathcal{R}T\ \ln a_1^{()} = -\tilde{v}_1\pi^{()} \cong \mathcal{R}T\ \tilde{v}_1\ \Sigma_j c_j^{()} \qquad j = 2,3,\ldots,m \qquad [4.5]$$

where $\pi^{()}$ is the *osmotic pressure* of the solution considered (the reader can easily confirm that equation 4.4 is equivalent to $-\tilde{v}_1\Delta\pi$). Similarly, the difference in chemical potential of the j-th species (other than water) is defined (Katchalsky and Curran, 1967) as:

$$\Delta\mu_j \cong RT\ (c_j^{(2)} - c_j^{(1)})\ /\ \bar{c}_j\ ;\ j \neq 1 \qquad [4.6]$$

This approximation relies also on the aforementioned assumptions coupled with the requirement that the concentration difference across the region of inhomogeneity is small, i.e. $\bar{c}_j \cong 1$. Let us note, however, that the error in supposing such a small difference even for a concentration ratio of $c_j^{(2)}:c_j^{(1)} = 2$ does not exceed 4% (10% for a ratio of 3; see Frömter [1983]).

Although these relations approach very crudely the reality, the scarcity of information available for such complex systems overshadows the inaccuracies introduced. We have to bear in mind that the utilization of the above relations does not rule out the possibility of replacing them by more formal definitions as additional information becomes accessible. In fact, further improvement to the descriptive accuracy of equations 4.4 and 4.6 can be obtained if one replaces the concentrations $c_j^{(1)}$ and $c_j^{(2)}$ by *effective* or *apparent concentrations*, $c_j^{(1)}(\mathrm{eff})$ and $c_j^{(2)}(\mathrm{eff})$, defined as $c_j^{()}(\mathrm{eff}) = \phi_j \nu_j c_j^{()}$, where ϕ_j is the *molar osmotic coefficient* of species j, generally construed as an *empirical correction factor* indicating the relative osmotic effect of that species, and ν_j is the number of dissociation products per mole of solute j. Since ϕ_j considers the fact that the solutes are *real* solutes and also takes into account the factors which cause the solvent to deviate from ideality, the range of application of equations 4.4 and 4.6 is therefore extended to *dilute* solutions of *real* solutes.

A point to be noted however about the use of osmotic coefficients is that they represent *corrections* necessary due to solute-solute and/or solute-solvent interactions only in the case of a particular *single* solute dissolved in a specific solvent. When more than one solute is present, interactions may occur between the different species which are not taken into account by the ϕ_j's. Predictions of $\Delta\mu_1$ for *multicomponent* solutions based on single solute coefficients are therefore only approximate.

An important outcome of this concept of apparent concentrations is that in the case of studies involving only *impermeable* solutes, if one has access to experimental measurements of the osmotic pressure, $\pi^{(\,)}$, of both intra- and extracellular solutions, it then becomes possible, using an extended form of relation 4.5 (see Nobel, 1983), i.e.:

$$\pi^{(\,)} = \mathcal{R}T\ \Sigma_j \phi_j \nu_j C_j^{(\,)} = \mathcal{R}T\ C^{(\,)}(\text{eff}) \qquad [4.7]$$

to calculate the apparent concentration, $C^{(\,)}(\text{eff})$, of a *fictitious solute* (representing the *lumped* osmotic properties of the species [often unknown] present in the solution) so as to make the calculated osmotic pressure equal to that which is actually observed. This apparent concentration can then be used advantageously in relations 4.4 and 4.6, for m = 2, to relieve the experimenter from the tedious task of obtaining not only a rather accurate compositional analysis but also good estimates of the osmotic coefficients pertaining to each species.

As can be seen, the requirement of ideality of the solutions can be released by the addition of the preceding improvements to the definitions of the thermodynamic forces (since it is now replaced by the less restrictive assumption that the osmotic coefficients stay constant throughout the osmotic exchanges). Still, it remains to discuss the question of the diluteness hypothesis.

Let us recall that the use of concentrations in all of the above relations relies on the assumption that the *volume* of both phases can be assimilated to the volume occupied by the *solvent*, i.e. $n_1^{()}\tilde{v}_1$, where $n_1^{()}$ is the number of moles of water in the solution and $n_1^{()} >>> \Sigma_j n_j^{()}$ for $j = 2,3,...,m$. Although this assumption is usually fulfilled in the case of the extracellular bath, this might not be the case for the intracellular solution. This problem can however be overcome, in practice, by introducing, as discussed by Levin, Ushiyama and Cravalho (1979), referring to the works of Levin, Cravalho and Huggins (1976, 1977), a *correction factor* often referred to as the *apparent cellular nonosmotic volume*, V_d, with the result that the effects of the intracellular solution nonideality and nondiluteness can be, to a large extent, "explained" by this factor. This apparent nonosmotic volume is usually assimilated to the volume of *cell solids*, including the volumes occupied by the intracellular solutes and colloids, the cellular structural material (wall and membranes), plus the volume of any water of hydration of the intracellular solutes and structural material which is *"bound"* too tightly to participate in osmotic phenomena. To that respect, V_d represents therefore the *"nonwater volume"* of the cell (we refer the reader to section 4.4 for a more detailed discussion on that factor).

The introduction of the nonosmotic volume concept implies that the cell *apparent "solution"* volume, V_s, that is, the volume which is *osmotically active*, does not correspond to the *total* cellular volume, $V^{(1)}$, and is, in fact, a fraction of that volume, namely:

$$V_s = V^{(1)} - V_d \qquad [4.8]$$

Equations 4.1 and 4.2 must therefore be rewritten in terms of the *effective* volume, V_s, rather than the compartment volume, $V^{(1)}$, which yields, assuming that V_d remains constant (see section 4.4):

$$dV_s/d\theta \equiv dV^{(1)}/d\theta = A\ \Sigma_k \tilde{v}_k J_k^{(1)} \qquad k = 1,2,\ldots,n \qquad [4.9]$$

and

$$dc_j^{(1)}/d\theta = [A/(V^{(1)}-V_d)]\ J_j^{(1)} - [c_j^{(1)}/(V^{(1)}-V_d)]\ dV^{(1)}/d\theta \qquad j = 2,3,\ldots,m \qquad [4.10]$$

Note that equation 4.8 has been used in these adjusted versions in place of V_s to account for the fact that measurements of $V^{(1)}$ and estimates of V_d are more readily obtainable experimentally than direct monitoring of the former volume.

The description of the transient behavior of the *cell membrane surface area* remains to be answered. It must be remembered that in the present analysis the "cell membrane" refers to the *total region of inhomogeneity* separating intra- and extracellular solutions. It is important to note that the dynamic model considers the cell as a simple osmotic system with *no intracellular compartmentalization*. The region of inhomogeneity is therefore assimilated "physically" to the *plasma membrane*; the permeability of which is assumed to represent the *total* of the resistances to be encountered during transfers between intra- and extracellular phases.

When considering the possible changes in the "membrane" surface area in response to osmotic stresses, one has to rely again on approximations. Indeed, since the information available on the mechanical and structural properties of membranes is scarce, a detailed description of the interfacial rearrangements pertaining to cellular volume changes is hardly attainable. The problem is further complicated as one tries, for complex geometries, to derive relations describing the dependence of the membrane surface area on the cellular volume. That is why it is customary to assimilate the form of the cell to a simple geometry (sphere, cylinder) and to either assume that the changes that occur are *isotropic*, that is, that the surface area of the membrane varies in a similar way in all directions, or simply to suppose that the membrane surface area remains constant, that is, that one assumes that, in *isotonic* conditions, the cell membrane is not fully distended and, depending on the nature of the bathing solutions, will either fold on itself or unfold until it reaches the point of lysis. In fact, as discussed by Levin and coworkers (Levin *et al.*, 1979), the assumption of constant surface area, rather than considering, for example, a 2/3rds power dependence of A on $V^{(1)}$ (as could be written for *isotropic* changes in *spherical* cells), has negligible impact upon the model performance and the values of the permeability estimates that could be derived from it.

4.1.2 Dimensional analysis

The above equations governing the movement of water and solutes into or out of a cell can be handled more efficiently if they are rewritten in *dimensionless form*. Hence, if we select the *initial* conditions of the system as *reference* values (subscript 0; see Levin *et al.*, 1979), we can define the following *dimensionless variables*[2]:

1. The reduced cellular volume:

$$\underline{V}^{(1)} = V^{(1)} / V^{(1)}_0 \qquad [4.11]$$

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[2] The *underscore*, "_", is used to differentiate a variable in *reduced* (dimensionless) form from its unit-dependent equivalent. The *slash*, "/", on the other hand, indicates a *modified* form that still carries some units (see List of Symbols).

2. The reduced cell membrane surface area:

$$\underline{A} = A / A_0 \qquad [4.12]$$

3. The reduced cellular nonosmotic volume:

$$\underline{V}_d = V_d / V^{(1)}{}_0 \qquad [4.13]$$

4. The j-th reduced concentration:

$$\underline{c}_j^{(1)} = c_j^{(1)} / c_j* \qquad [4.14]$$

where c_j* is the reference concentration for species j, defined as:

$$c_j* = .5\ (c_j^{(1)}{}_0 + c_j^{(2)}{}_0) \qquad [4.15]$$

5. The j-th reduced thermodynamic force:

$$\Delta\underline{\mu}_j = \Delta\mu_j / \mathcal{R}T \qquad [4.16]$$

where T is the absolute temperature of the system.

6. The ij-th modified macroscopic phenomenological coefficient:

$$\underline{L}_{ij} = \varnothing\, L_{ij} \text{ for } i=j \text{ and } i \neq j \text{ forms} \qquad [4.17]$$

where $\varnothing$ is a *normalization factor* defined as:

$$\varnothing = A_0\, \mathcal{R}T* / V^{(1)}{}_0 \qquad [4.18]$$

where T* is an arbitrary *reference* temperature chosen identical to the reference temperature appearing in equations 3.9 and 3.10, and finally,

7. The modified transmembrane flux of species j:

$$\mathcal{J}_j^{(1)} = (\varnothing/\mathcal{R}T)\ J_j^{(1)} \qquad [4.19]$$

These variables are then inserted in the model equations (equations 4.9 and 4.10) leading, after proper manipulations, to their equivalent dimensionless forms, namely:

1. The cell volume change:

$$d\underline{V}^{(1)}/d\theta = \underline{A}\ (\mathcal{R}T/\mathcal{R}T*)\ \Sigma_k \hat{v}_k \mathcal{J}_k^{(1)}$$
$$k = 1,2,\ldots,n \qquad [4.20]$$

and

2. The change in the intracellular concentration of species j:

$$d\underline{c}_j^{(1)}/d\theta = [\underline{A}/(\underline{V}^{(1)}-\underline{V}_d)]\ (\mathcal{R}T/\mathcal{R}T*)\ (\mathcal{J}_j^{(1)}/c_j*) -$$
$$[\underline{c}_j^{(1)}/(\underline{V}^{(1)}-\underline{V}_d)]\ d\underline{V}^{(1)}/d\theta$$
$$j = 2,3,\ldots,m \qquad [4.21]$$

For their part, the modified fluxes are evaluated using the dimensionless forms of relations 2.13, 4.4 and 4.6, that is:

$$\mathcal{J}_j^{(1)} = \Sigma_i Ł_{ij} \Delta\underline{\mu}_i \ ;\ i,j = 1,2,\ldots,m \qquad [4.22]$$

where

$$\Delta\underline{\mu}_1 = -\ \hat{v}_1\ \Sigma_j c_j*\ (\underline{c}_j^{(2)} - \underline{c}_j^{(1)})$$
$$j = 2,3,\ldots,m \qquad [4.23]$$

and

$$\Delta\underline{\mu}_j = (\underline{C}_j^{(2)} - \underline{C}_j^{(1)}) / \bar{\underline{C}}_j \; ; \; j = 2,3,\ldots,m \qquad [4.24]$$

where $\bar{\underline{C}}_j$ is the dimensionless form of the average concentration of species j across the membrane, that is:

$$\bar{\underline{C}}_j = .5\ (\underline{C}_j^{(1)} + \underline{C}_j^{(2)}) \qquad [4.25]$$

Finally, this system of differential equations has to be solved for the following initial and boundary conditions, namely:

1. For $\theta = 0$

$$\text{a)}\ \underline{C}_j^{(1)} = \underline{C}_j^{(1)}{}_o \text{ and } \underline{C}_j^{(2)} = \underline{C}_j^{(2)}{}_o$$
$$j = 2,3,\ldots,m \qquad [C.1]$$

$$\text{b)}\ \underline{A} = 1 \text{ and } \underline{V}^{(1)} = 1 \qquad [C.2]$$

and

2. For $\theta > 0$

$$\underline{C}_j^{(2)} = \underline{C}_j^{(2)}{}_o \qquad [C.3]$$

Condition C.3 corresponds to the assumption that the extracellular solution volume is such that its composition is virtually unaffected by the transmembrane exchanges. Note also that the time variable, θ, is *not* in dimensionless form.

4.2 The nature of the observed data

The study of cell membrane permeability began with purely qualitative or at best semiquantitative descriptions of the behavior of various penetrating substances. Later workers have considerably extended our knowledge of the osmotic response of cells to certain solutes by following, for example, the exact course of the volume changes over long periods of time of those cells exposed to various osmotic solutions. In spite of the large amount of information which accumulated on permeability phenomena, it was not until Kedem and Katchalsky's monumental work (Kedem and Katchalsky, 1958) that these studies could be analyzed and cast in the form of a self-consistent and general set of equations.

The major impact of the Kedem-Katchalsky relations (equations 2.18, 2.19 and 2.20), apart from *standardizing* the terminology of osmometric studies, was to define *explicitly* the nature of the experiments to be carried out to obtain the *pertinent* data required to estimate the permeability coefficients. For example, referring to equation 2.18, we can see that the hydraulic permeability, Lp, can be estimated by either measuring Jv for a given ΔP when all $\Delta\pi_t$ *and* $\Delta\pi_k$ are null, that is:

$$Lp = (Jv/\Delta P)|_{\Delta\pi_k,\Delta\pi_t=0} \qquad k = 2,3,\ldots,n \text{ and } t = n+1,n+2,\ldots m \qquad [4.26]$$

or, alternatively, when ΔP and all $\Delta\pi_k$ are zero, by measuring Jv for given $\Delta\pi_{i}$, that is:

$$Lp = (Jv/\Delta\pi_{im})|\Delta P,\Delta\pi_{k}=0$$
$$k = 2,3,\ldots,n \qquad [4.27]$$

where $\Delta\pi_{im}$ refers to a *fictitious* impermeable species regrouping all impermeable solutes on both sides of the membrane (see section 4.1.1). The latter technique consists in suddenly placing the cell in, say, a hypertonic solution of an *impermeable* solute and then recording the time course of shrinkage. Knowledge of the *initial* value of the cell volume change and of the cell membrane surface area allows one to determine *Lp* through a relation equivalent to equation 4.20. In a similar way, experiments can be devised from the basic relations to determine the other permeability coefficients (details can be found elsewhere [House, 1974; Macey, 1979]).

The above approach requires, therefore, the performance of *one independent* experiment for each coefficient to be estimated. With the advent of more sophisticated techniques, however, certain workers have looked at the possibility of using the *entire* time course of the osmotic volume change to determine one or more coefficients at a time, instead of restricting the analysis to the initial phase of the osmotic transient (Hempling, 1967; Sha'afi, Rich, Mikulecky and Solomon, 1970; Stusnick and Hurst, 1972). This procedure is based on the assumption that the transient behavior of the cell under study can be described by dynamic models similar to the relationships introduced in the previous section. This is not too restrictive since the parameters estimated employing these relations reflect implicitly the appropriateness of the models which, in the context of *comparative* studies, becomes irrelevant. In fact, as pointed out in section 2.2.4, the major restriction comes from the intrinsic interdependence of the Kedem-Katchalsky coefficients which, strictly speaking, renders invalid the above approach for *simultaneously* estimating more than one coefficient. It appears, therefore, that the use of the entire transient curve is applicable only in conjunction with the more fundamental form of the phenomenological coefficients which, as we have established, are truly independent.

One might wonder, on the other hand, if the macroscopic phenomenological coefficients could be estimated from independent experiments of the form described by equations 4.26 and 4.27. Referring to equation 3.1, it can be seen that, theoretically, if one can control the chemical potentials of the species and measure independently their fluxes, estimates of the macroscopic coefficients could be obtained. However, due to the nature of the chemical potentials which are governed by the Gibbs-Duhem relation (equation 2.15), the experimental conditions are difficult to achieve, especially in the case of estimating the cross-coefficients.

Yet, one way of achieving the experimental requirements described by the formal definition of the coefficients is to carry the measurements with the help of *radioactive tracer* substances. Indeed, since they can be detected in extremely low concentrations, they can be added in minute amount in a solution without really disturbing the thermodynamic properties of the system. Therefore, if we assume that both labelled and unlabelled isotopes do not differ in their transport behavior, that is, apart from being marked, a labelled molecule is identical to its unlabelled counterpart, it is then possible, while maintaining a cell in equilibrium with a given osmoticum, to add the labelled species to the bath containing unlabelled isotopes and to monitor the flux of the marked molecules in response to the slight gradient just induced. The main advantage of this technique resides in its conformity with the limiting process described by equation 3.1. There are, nevertheless, some restrictions imposed on the analysis of such measurements and the interested reader should consult Frömter (1983) and Sauer (1978) for more details.

Among the two techniques outlined in this section, the former, the observation of the transient cell volume during an osmotic stress, seems, by far, the method selected by a majority of workers for studying permeability phenomena (Macey, 1979). Furthermore, the advent of microcomputers coupled with the use of increasingly sensitive monitoring devices provide the researcher with high quality measurements. As a consequence, we will now examine the requirements of the statistical theory as applied to the analysis of this type of experimental data, an obvious prerequisite which, in our opinion, is often bypassed by a majority of workers.

4.3 Statistical analysis of the experimental data

It seems that most researchers have overlooked an important fact related to the use of theoretical models not only to describe the transient behavior of cells exposed to osmotic stresses but also to estimate, from these relations, membrane permeability coefficients. One must be aware that these equations have been derived for a *single* cell and consequently are not necessarily applicable to a *population* of cells in a suspension (Dainty, 1976). As a matter of fact, most of the experimental techniques monitor the volume changes with time of a *population* of cells after exposure to a solution containing permeable and/or impermeable solute(s) (Stusnick and Hurst, 1972; Hempling, 1967; Levin, 1979; McGann, Turner and Turc, 1982; Galey, Gillon and Murphy, 1980). The differential equations are then used to fit the experimental curves using nonlinear regression techniques and the fitted permeability coefficients are said to be estimates of the permeability parameters of the observed cells.

This is a rather premature conclusion. It is important to understand that the latter statement is strictly applicable only in the situation where the studied cells are *all alike*, in which case the behavior of the suspension as a whole is merely a *macrorepresentation* of the behavior of each individual. Equations 4.20 and 4.21 can then be directly applied to estimate the coefficients.

In reality, however, it is most likely that the cells will exhibit a certain distribution in their physical characteristics (Jay and Rowlands, 1975; Saari and Beck, 1974) and, although they might have the same composition at the onset of the experiment, their behavior will not be the same during the osmotic process (see Mazur, 1981; Jay and Rowlands, 1975). It is therefore indispensable to know what is the nature of the observation made prior to the statistical analysis to ensure that experimental and theoretical data are truly comparable.

4.3.1 Restrictions imposed by the experimental techniques used

As noted previously, most of the experimental techniques monitor the volume changes with time of a population of cells exposed to osmotic solutions. Two types of procedures are generally used:

1. *Direct microscopic observation* of randomly selected population members *throughout* the experiment (Stusnick and Hurst, 1972; Levin *et al.*, 1979; Diller and Bradley, 1984) and
2. *Indirect monitoring* of volume changes of population

samples through the use, for example, of a particle sizer (McGann *et al.*, 1982; Rule, Law, Kruuv and Lepock, 1980) or optical devices (Galey *et al.*, 1980).

As we can imagine, the nature of the observed transients differs for each type. For instance, in the first case, we have access to the behavior of *individuals*. Though these individuals might initially be of different sizes or selected to be relatively similar, equations 4.20 and 4.21 (or their equivalents) are generally used to generate theoretical volume changes that are directly compared to each set of experimental data. Pooled estimates of the permeability coefficients are then calculated (with their standard errors) from this sample of coefficient values. There are, however, two important issues associated with this approach.

Firstly, one should be concerned by the inherent violation of the requirement of *independence of the random error* due to the *time correlation* between the observations. Indeed, each "set" of experimental data corresponds to observing the transient changes of the *same* individual over a given period of time, a situation which is generally seen as a major problem in regression. Although, in the case of *linear* models, it is usually possible to "*correct*" the relations by means of some *transformations* to uncorrelate the experimental data (Kleinbaum and Kupper, 1978), it is, for nonlinear cases, practically impossible to find a suitable modification, especially if one deals with *differential equations* with *unknown* solutions. Secondly, based on the same arguments, there is no way of determining the exact *pooling* procedure (arithmetic average, geometric average, etc.) necessary to estimate the permeability coefficients. We can therefore state, in view of the complexity of the statistical treatment required to analyze this type of data, that this experimental procedure leads to estimates which carry intrinsically these unknowns.

In the second case, on the other hand, the techniques do not allow continuous observation of *definite* cells and force the experimenter to continuously monitor the volume change of *samples* of relatively large number of cells. Although *each* cell might be measured, the sampling process is purely random and allows only the estimation of the mean sample volume and possibly computation of the variance of the sample at each sampling time. The experimental data are, as we can see, *average* values and as such are a function of the population distribution.

Let us assume that the permeability coefficients are independent of the geometric parameters of the cell. This is a reasonable assumption, especially for individuals that have reached the same degree of maturity since, as the properties and structure of the membrane system are genetically determined, it is expected that the behavior of their membranes will be similar. Let us further assume that the initial population distribution is known (most likely a normal distribution) and that the sample size for each measurement is big enough to ensure that the distribution of the sample approximates closely to the population distribution.

As we can see, the experimental data are thus analogous to the volume changes of a *hypothetical* cell having the *average* properties of the population used for the experiment, the estimated variances being a measure of the exactness of this hypothesis. The questions then are: "How can we draw conclusions as to the permeability characteristics of that population of cells from such data since the differential equations describing the transport phenomena (equations 4.20 and 4.21) have been derived theoretically for a single cell?", "Can we directly fit them to the curve generated with *average* volumes?" and, if yes, "How do the obtained parameters compare to the average values generated by the first technique?". Further, "Can we use the fact that each average volume is the mean response of a sample from the cell population to make inferences as to the dependence of the permeability characteristics on the population properties?".

There is a high probability that the response of any cell will be species and age dependent, which is a precondition for a cell suspension to be *homogeneous*. Provided that the cell suspension under investigation satisfies the above condition, we can legitimately apply a suitable *weighted* nonlinear regression technique to fit the experimental data and use equations 4.20 and 4.21 rigorously to generate the predicted values. Let us recall that a weighted regression technique uses the inverse of the estimated variance of each data point as "weights" to force the algorithm to literally anchor the solution to the most accurate observations, that is, the points that bear the "heaviest" weights. This process is particularly crucial in view of the drastic changes that occur in the early stages of the cell response which call for an accurate monitoring of the cell behavior. We will examine this technique in more detail in section 4.3.3.

The condition of homogeneity cannot be overemphasized. The validity of the regression treatment is limited basically to measurements in which the only source of variation can be attributed to the population distribution. The observed scatter should not be due to cells that are not physically sound.

The major advantage over the first type of data is that it gives access to a better measure of *population variation* of the permeability coefficients. This result would be achieved in the first case only if the selected cells were a true representation of the population (an assumption which is greatly dependent on the sample size) and if the measurements were truly uncorrelated. The second type of procedure, on the other hand, enables the experimenter to monitor the changes from samples which are statistically closer to a true representation of the population under investigation since the measurements are made practically on thousands of individual cells. Furthermore, in the eventuality where the population distribution is unknown and/or nonnormal and the access to estimates of the sample variances is impossible, the use of *replicated* sample means, by virtue of the *central limit theorem*, "normalizes" the experimental data. Let us recall that the probability density of sample means computed from nonnormal random variables is more *symmetric* than the underlying distribution and has less dispersion.

This normalization is particularly crucial in the context of parameter optimization based on the *least-squares minimization* criterion since the validity of the whole treatment relies on the assumption that the fitted data are normally distributed (see section 4.3.3). We can therefore expect that the values of the coefficients estimated from the latter treatment will depict more accurately the population behavior, especially in view of the fact that we are, in that case, in full control of the various underlying requirements pertaining to the statistical theory.

We will now turn our attention to the *design* of the experiments necessary to acquire the information pertinent to the estimation of the cellular permeability coefficients.

4.3.2 Restrictions based on the nature of the information

As discussed in Chapter 3, the macroscopic phenomenological coefficients are strongly influenced by the variation of the intensive and extensive properties of the bounding phases. These coefficients are therefore, in the *Onsager* form (L form), *point* functions, that is, their values change along with the state of the system. However, in terms of optimized parameters, we are more interested in coefficients that describe the membrane properties for the *entire* experimental range than in local values. That is why we have introduced the *extended* forms of the macroscopic phenomenological coefficients (equations 3.9 and 3.10) to take into account, among other things, the dependence of these coefficients on the temperature and composition of the phases, hoping that, by *empirically* breaking down the Onsager coefficients in their *constitutive* parts, we will obtain parameters which will be relatively independent of the variation of the thermodynamic properties of the system. We have therefore to realize that the optimization procedure will search for the values of the *macroscopic permeability coefficients*, Þ_{kk} and Þ_{jk} (or their temperature-independent form, Þ°_{kk} and Þ°_{jk}, and the associated *apparent transfer energies*, A_{kk} and A_{jk}), which will explain as accurately as possible the transient behavior of the cells and, as a consequence, the macroscopic properties of their membranes. They are the coefficients which, during the search, are altered and used (through relations 3.9 and 3.10) to compute the local values of the L's appearing in equation 2.13 or, in modified form, in equation 4.22.

This approach has an important bearing upon the nature of the information required to perform the optimization.

Firstly, let us recall that the whole theoretical development presented in the previous sections is based on the analysis of transmembrane transport phenomena in an *isothermal* environment (see section 2.2.3), which therefore requires that all experiments be carried out at *constant* temperature since the possible influence of temperature gradients across the region of inhomogeneity is not considered in the entropy production (equation 2.12). Hence, any temperature dependence study of the macroscopic permeabilities must originate from analyzing *isothermal* measurements performed at various temperatures, rather than from experiments where the temperature is allowed to vary. Secondly, a temperature sensitivity of the coefficients can be legitimately accessed only if the experimental design provides one more *independent* observation than the number of parameters to optimize which, in our case, requires data coming from *at least* 3 isothermal studies.

In terms of concentration variation, the evolution of the system during osmotic changes ensures that a certain range of concentrations is available for study. One can nevertheless complete the data by performing experiments with different osmoticum compositions to cover a wider range of concentration gradients experienced by the cells. It must be emphasized, though, that the "*predictive*" power of the model associated with the optimized parameters is restricted to the region covered by the experiments. Any attempt to use estimates to predict beyond the explored range might lead to important discrepancies. It is therefore in one's interest to design the experiments to encompass the widest environmental variation as possible.

4.3.3 Estimation of the variances of the experimental data: the weighted least-squares procedure

The experimental procedure adopted in the present study is based on a technique developed by McGann *et al.* (1982) which permits rapid measurement of *average* volumes of cells exposed to osmotic solutions (details can be found in section 4.4). These mean volumes correspond to averaging (arithmetic average) volumetric measurements performed on approximately 170 cells over an interval of 100 milliseconds. The latter process is necessary due to hardware limitations which render difficult the storage of the individual values for the entire time span covered by the experiments. However, a counter allows the experimenter to know the exact number of cells averaged at each sampling time and consequently this datum is accessible along with its associated mean. The question arising therefore is: "How can one then make the most efficient use of this information?". That is what we will now examine.

As pointed out by Kleinbaum and Kupper (1978), any regression analysis is based on certain statistical assumptions, of which two have already been mentioned, namely, that the observed values (the *dependent* variable values) are *statistically independent* of one another and that, for any fixed value of the *independent* variable(s), the observed data belong to a *normal* population. It is important to emphasize that, if the latter assumption is not *badly* violated, the conclusions reached by a regression analysis, assuming normality, will generally be reliable and accurate.

A third statistical assumption, which is generally tacitly made, assumes that the *variance* of the dependent variable is the *same* for any value of the independent variable(s) (assumption of *homoscedasticity*). However, as demonstrated by Toupin, Le Maguer and McGregor (1983), violation of this assumption can lead to serious errors in parameter estimation. Hence, one should, whenever possible, use a *weighted least-squares* procedure to properly handle data for which the assumption of homogeneity of variance is doubtful.

This method of analysis is a modification of standard regression analysis procedures and is designed for the situation in which a regression model is to be fitted to a set of data for which the assumptions of homoscedasticity and/or independence do not hold. We will briefly describe here the approach for dealing with variance heterogeneity. We refer the reader to Draper and Smith (1981) for a discussion of the general method of weighted regression which incorporates the nondependence situation.

When the variance of the observed data varies for different values of the independent variable(s), weighted least-squares analysis can be used, provided these variances are *known*, or can be assumed to be of the following form:

$$\sigma_i^2 = \sigma^2 / W_i \qquad [4.28]$$

where σ_i^2 is the variance for the i-th observation on the dependent variable, σ^2 is the *population* variance, and W_i is a *known "weight"*. The methodology then involves determining the model parameters which minimize the expression:

$$\Sigma_i W_i (Y_i - \hat{Y}_i)^2 \ ; \ i = 1,2,\ldots,X \qquad [4.29]$$

where the summation runs for the X fixed values of the independent variable(s) at which an observation on the dependent variable Y was made and W_i is the i-th weight given either by $1/\sigma_i^2$ when σ_i^2 is known, or is exactly the W_i in relation 4.28 when that form applies. Finally, $\hat{Y}_i$ is the value of the dependent variable Y *predicted* from the model equation at the i-th value of the independent variable(s). The major difficulty with using equation 4.29 resides in the fact that the above variances are rarely known. One must therefore rely on *estimates* in order to apply the technique. This, however, can be achieved *only* if *replicated* observations are available.

It can be shown (Mendenhall and Scheaffer, 1973) that an *unbiased* estimate of the variance of a given population can be calculated from a *sample* of that population using the following expression:

$$\hat{\sigma}_i^2 = [\Sigma_j (Y_{ij} - \bar{Y}_i)^2]/(k_i - 1)$$

$$j = 1,2,\ldots,k_i \qquad [4.30]$$

where $\hat{\sigma}_i^2$ is the estimated variance of the population of Y's sampled at the i-th independent variable(s) value(s) and $\bar{Y}_i$ is the mean of the sample k_i replicated observations given by:

$$\bar{Y}_i = (1/k_i)\,\Sigma_j Y_{ij}\ ;\ j = 1,2,\ldots,k_i \qquad [4.31]$$

This estimate of the *accuracy* of the i-th set of observations, as being *representative* of its population, is then used advantageously to force the fitted regression line to go through the well-defined distributions (where the variances are small) and to consider with less importance the distributions which are too spread (where the variances are large). One should now realize the importance of *replicated measurements*, since this is the only possible source of information to estimate the required weights.

Let us now see how we can reformulate the preceding relations in a form suitable for analyzing our experimental data. As discussed in the introductory statements, the dependent variable values are, in our case, *average* volumes computed from samples of cells measured during time intervals of about 0.1 second. One must be aware however that these cells are not measured simultaneously, but are in fact gauged *individually*, each 600 µs, allowing, as we have stated, about 170 cells to be sampled during that 100 milliseconds time interval. These observations are not therefore, in the true sense of the word, "replicates" since they are not measured at the same time. We can nevertheless consider them as *approximate repeats* if we assume that *during the interval* the behavior of the cells is essentially *independent* of time. In such a case, as pointed out by Draper and Smith (1981), we can use these pseudo-repeats as though they were repeat measurements and evaluate an approximate population mean and variance from them. Unfortunately, we do not have access to the individual values, nor can we compute the sample dispersion. We must therefore rely exclusively on the means of the intervals and on the number of individuals used to compute them to obtain the information necessary to perform the weighted least-squares analysis.

The above problem can be solved if we carry out *replicate runs*, that is, if we generate *replicates* of average volumes at each sampling time. Let the subscript i indicate the *time* scale position (independent variable value, θ) and the subscript j indicate a given average volume (dependent variable value). Furthermore, let n_{ij} represent the number of individuals in the ij-th sample, whereas m_i indicates the number of replicated means available at the i-th time position. Equation 4.29 is then rewritten as follows; the problem being to determine the set of modified permeability coefficients which *minimizes* the sum of squares, given by:

$$\Sigma_i \Sigma_j W_{ij} (\bar{Y}_{ij} - \hat{Y}_i)^2$$

$$i = 1,2,\ldots,\theta \text{ and } j = 1,2,\ldots,m_i \qquad [4.32]$$

where $\bar{Y}_{ij}$ is the j-th average volume measured at the i-th time point. $\hat{Y}_i$ and W_{ij} are, respectively, the predicted cell volume (calculated using the model equations) and the j-th weight (estimated at the same time value).

Let us assume that, at any given time point, the samples used to estimate the average volumes come from the *same parent population* with mean μ_i and variance σ_i^2, i.e.: $\mu_{i1} = \mu_{i2} = \ldots = \mu_i(m_i) = \mu_i$ and $\sigma_{i1}^2 = \sigma_{i2}^2 = \ldots = \sigma_i^2(m_i) = \sigma_i^2$. The value of the ij-th weight is then given by definition by:

$$W_{ij} = [\text{estimate of } \mathrm{Var}(\bar{Y}_{ij})]^{-1} \qquad [4.33]$$

which can be shown (Mendenhall and Scheaffer, 1973) to be equivalent to:

$$W_{ij} = [\text{estimate of } \sigma_i^2/n_{ij}]^{-1} \qquad [4.34]$$

If we now suppose that the variances of the populations of individuals are all equal for the entire experimental time range, θ, that is, $\sigma_1^2 = \sigma_2^2 = \ldots = \sigma^2(\theta) = \sigma^2$, then the ij-th weight is defined as:

$$W_{ij} = n_{ij} \qquad [4.35]$$

Equation 4.35 simply states that, if one assumes variance homogeneity, the *number of cells* can be used directly as an index to "quantify" the confidence attached to the ij-th experimental mean. On the other hand, if homoscedasticity does not hold, that is, $\sigma_1^2 \neq \sigma_2^2 \neq \ldots \neq \sigma^2(\theta)$, then the ij-th weight becomes a quantity which will depend not only on the time position but also on the number of individuals in the ij-th sample, that is:

$$W_{ij} = [\hat{\sigma}_i^2/n_{ij}]^{-1} \qquad [4.36]$$

Having established the forms that the weights can take, it remains to describe how the terms appearing in these definitions can be evaluated. In terms of the number of individuals belonging to the ij-th mean, i.e. n_{ij}, this poses no problem since this information is part of the data generated by the experimental technique. The variance estimate, $\hat{\sigma}_i^2$, for its part, is computed using the following expression:

$$\hat{\sigma}_i^2 = [\Sigma_j n_{ij}(\bar{Y}_{ij}-\bar{\bar{Y}}_i)^2]/(m_i-1)$$

$$j = 1,2,\ldots,m_i \qquad [4.37]$$

where $\bar{\bar{Y}}_i$ is the *weighted grand mean* of the i-th set of observed averages, given by:

$$\bar{\bar{Y}}_i = [\Sigma_j n_{ij}\bar{Y}_{ij}]/\Sigma_j n_{ij}$$

$$j = 1,2,\ldots,m_i \qquad [4.38]$$

If, on the other hand, one had access to the volume of each individual, i.e. Y_{ijk}, the relation to be employed would take a form similar to equation 4.30, namely:

$$\hat{\sigma}_i^2 = [\Sigma_i\Sigma_k(Y_{ijk}-\bar{Y}_{ij})^2]/(\Sigma_j n_{ij}-m_i)$$

$$j = 1,2,\ldots,m_i \text{ and } k = 1,2,\ldots,n_{ij} \qquad [4.39]$$

Both forms are valid *unbiased* estimators of the i-th population variance and, in an *analysis of variance (ANOVA)* context, are often referred to as the *treatment mean square* (equation 4.37) and the *error mean square* (equation 4.39), respectively (Kleinbaum and Kupper, 1978). Hence, inserting relation 4.37 in relation 4.36, leads to the following definition of the ij-th weight:

$$W_{ij} = \{[\Sigma_j n_{ij}(\bar{Y}_{ij}-\bar{\bar{Y}}_i)^2]/[(m_i-1)n_{ij}]\}^{-1} \qquad [4.40]$$

Equation 4.37 can also be applied to estimate the variance of measurements performed in *isotonic* solutions for *calibration* purposes. Since in that case the volume of the cells is *constant* in time, the whole data set is equal to having a series of m means, $\bar{Y}_j(\text{iso})$, measured at a single time point. If individual measurements, $Y_{jk}(\text{iso})$ were available, the mean of that sample of the population, $\bar{\bar{Y}}(\text{iso})$, would be calculated as follows:

$$\bar{\bar{Y}}(\text{iso}) = [\Sigma_j \Sigma_k Y_{jk}(\text{iso})]/\Sigma_j n_j$$
$$j = 1,2,\ldots,m \text{ and } k = 1,2,\ldots,n_j \qquad [4.41]$$

which is equivalent to:

$$\bar{\bar{Y}}(\text{iso}) = [\Sigma_j n_j \bar{Y}_j(\text{iso})]/\Sigma_j n_j$$
$$j = 1,2,\ldots,m \qquad [4.42]$$

where n_j is the number of individuals in the j-th mean. The variance of $\bar{\bar{Y}}(\text{iso})$ is given by definition by:

$$\sigma^2[\bar{\bar{Y}}(iso)] = \sigma^2 / \Sigma_j n_j \qquad j = 1,2,\ldots,m \qquad [4.43]$$

where σ^2 is the variance of the population of individuals. This population parameter is approximated using equation 4.37, giving:

$$\hat{\sigma}^2 = \{\Sigma_j n_j[\bar{Y}_j(iso)-\bar{\bar{Y}}(iso)]^2\}/(m-1) \qquad j = 1,2,\ldots,m \qquad [4.44]$$

which yields the following estimator of the variance of the population mean:

$$\hat{\sigma}^2[\bar{\bar{Y}}(iso)] = \{\Sigma_j n_j[\bar{Y}_j(iso)-\bar{\bar{Y}}(iso)]^2\}/[(m-1)\Sigma_j n_j] \qquad j = 1,2,\ldots,m \qquad [4.45]$$

Before concluding this section, let us point out that the use of *means* instead of individual values should not be seen as a limitation. In fact, as discussed section 4.3.1, it turns out to be, if anything, an advantage since these samples of means are, by way of the *central limit theorem*, closer to a normal distribution that the subjacent population. Let us also add that the experimental procedure adopted in this work ensures *perfect independence* of the observed data since the sampled individuals are *removed* from the osmotic bath as they are measured (the *density* of the population is however not affected since the volume of the osmoticum is reduced accordingly). Finally, let us note that the use of the weighted least-squares analysis not only relieves the experimenter from assuming homoscedasticity but also allows the possibility of characterizing the degree of certainty that can be attached to the data. We can therefore be relatively confident that, in its present form, the information accessible for treatment respects the basic requirements of the statistical theory.

Chapter 5 will be devoted to presenting some examples of the application of the preceding analysis to the estimation of membrane permeability coefficients. Before moving to that section, though, let us describe briefly the experimental methods used and present the results of preliminary calculations necessary to perform the parameters estimation.

4.4 Methods and experimental procedures

The cells used in this work were *granulocytes* isolated by density gradient centrifugation from the peripheral blood of normal human donors using a technique described by Segal, Fortunato and Herd (1980). Briefly, defibrinated whole blood was sedimented with dextran to remove erythrocytes, and the leukocyte-rich suspension was layered on a continuous density gradient of Percoll (mean density = 1086 kg m^{-3}). Centrifugation at 800g for 20 minutes produced three well-separated bands, namely: mononuclear leukocytes, granulocytes and erythrocytes. The granulocyte layer was recovered and washed in phosphate-buffered saline (PBS) with 0.6% bovine serum albumin. This method routinely provided a population of cells consisting of about 95% granulocytes, capable of phagocytosis of zymosan particles, reduction of nitro blue tetrazolium and chemiluminescence.

The principle of *electronic sizing of particles* was used in the measurement of cell volumes, the magnitude of the pulses from the amplifier of an electronic particle counter, such as the Coulter ZBI used in this study, being proportional to the volume of the cell traversing the orifice of the counter (Adams, Voelher and Gregg, 1967). An electronic signal-processing interface designed by McGann and coworkers (McGann *et al.*, 1982) was used in combination with a microcomputer system to allow rapid volume data acquisition. The interface circuit involves a peak detector, sample-and-hold amplifier and a fast analog-to-digital converter, along with appropriate hand-shaking circuitry which permits communication between the interface and the microcomputer. Briefly, the volume of each cell traversing the counter orifice is measured. The digitalized reading is then added to the content of a given memory location and the memory location corresponding to the number of cells gauged is incremented. At each clock interrupt, i.e. at each sampling interval, another summation is initiated in a different memory location and its cell counter set to zero. The process continues for the duration of the experiment. A separate program is used concurrently to read the data from memory and to compute the average readings by dividing the stored summations by their associated number of cells. This program can also provide a visual representation of the data on a video terminal during the period of acquisition, or transfer the data to disk for later analysis.

Aliquots of 50 μl of isolated granulocyte suspension were injected into stirred osmotic solutions (≅10 ml) of known composition and average cell volumes evaluated as a function of the time of contact. The cell concentration was adjusted so that, for sampling intervals of 1 second, between 2,000 and 4,000 cells could be measured for each determination of an average volume. Beads of known volume were used for calibration. It was assumed that the responses of the cells and the beads during volumetric gauging were identical, therefore allowing direct calculation of absolute volumes. The average calibration factor was 0.511 ± 0.004 (*MEE*)[3] for settings of 1/amp = 1 and 1/gain = 1. It was assumed that this factor was temperature independent, which is reasonable since neither the density of the bathing solution nor the density of the beads can be expected to change noticeably in the temperature range used in this work. This assumption is supported by the fact that, using this factor, isotonic measurements performed at temperatures varying from 5°C to 30°C (by steps of 5 C°) led to estimates of mean cellular volumes which did not show any temperature dependence. All measurements were therefore lumped and a mean corpuscular volume of 3.45 ± 0.01 (*SDM*)[4] x 10^{-14} m^3 was found for our sample of human granulocytes, which compares extremely well with an estimate of 3.46 ± 0.03 (*SDM*) x 10^{-14} m^3 reported by Hempling (1973).

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[3] The *maximum estimated error* (*MEE*) is the maximum error attached to the datum as a result of applying the principles of *propagation of uncertainties in functions* (Baird, 1962).

[4] *SDM*: standard deviation of the mean (standard error).

The evaluation of the *apparent cellular nonosmotic volume*, $\underline{V}_d$ (see section 4.1.1), was carried out assuming that the *equilibrium* volume of cells exposed to an osmoticum containing an *impermeable* solute obeyed the *Boyle-Van't Hoff* relation, i.e. that the volume of any cell at equilibrium varied *linearly* with the inverse of the osmotic pressure of the bathing solution, π, namely:

$$\underline{V}^{(1)}{}_{\infty} = \underline{V}_d + \underline{V}_s{}^{\circ}(\pi^{\circ}/\pi) \tag{4.46}$$

where $\underline{V}^{(1)}{}_{\infty}$, $\underline{V}_d$, $\underline{V}_s{}^{\circ}$ and π° are, respectively (see section 4.1.2), the reduced form of the cellular volume at equilibrium, the apparent nonosmotic volume, the *initial* cell "solution" volume, and the initial osmotic pressure of the cell solution (isotonic condition).

A plot of $\underline{V}^{(1)}{}_{\infty}$ as a function of π°/π should therefore yield a straight line of slope $\underline{V}_s{}^{\circ}$ and intercept $\underline{V}_d$. The apparent nonosmotic volume is consequently assimilated to the cell volume theoretically attained in an external solution of *infinite* osmotic pressure. For such an infinite π, all the internal water would be removed and the volume, which is actually obtained by *extrapolation*, would be the volume of the *nonaqueous* cell components. However, some uncertainty exists for water that is tightly bound to proteins and other substances. Part of this water presumably remains bound even at the *hypothetical* infinite osmotic pressure considered here. $\underline{V}_d$ should therefore be seen more as a factor that is useful for the *empirical* description of $\underline{V}^{(1)}_\infty$ on π rather than having a direct physical meaning (Macey, 1979).

An important consequence of this experimental determination of $\underline{V}_d$ is that since the Boyle-Van't Hoff relation (as represented by equation 4.46) is derived assuming among other things *diluteness* of the intracellular solution (see Nobel, 1983), the value of $\underline{V}_d$ reflects therefore *inherently* the effects of any departure from that hypothesis. Furthermore, since the response of the cells is dependent on the osmotic behavior of the intracellular solution, $\underline{V}_d$ should also describe the effects of strong deviations from ideality. It then follows that: (1) if the experimental points used to obtain this estimate are within the range of osmotic pressures to be used in the osmometric studies, and (2) the changes in volume of the studied cells obey reasonably well the above relation, one can legitimately use equations 4.4 and 4.6 to approximate the thermodynamic forces present in the system, provided that $\underline{V}_d$ is introduced as a correction factor in the differential equations describing the behavior of that population (equations 4.9 and 4.10 or 4.20 and 4.21 [in reduced form]). Another obvious consequence is that the fitting procedure yields a parameter which is entirely independent of $V^{(1)}$ and of the intra- and extracellular osmolalities, thus reinforcing the empirical nature of $\underline{V}_d$. Equation 4.46 needs however to be rearranged to lead to a valid statistical analysis.

As a close look at the above relation reveals, the intercept and the slope are highly *correlated* since, by definition (see equation 4.8):

$$\underline{V}_s^\circ = 1 - \underline{V}_d \qquad [4.47]$$

Hence, a modified version of equation 4.46 was adopted which yields an uncorrelated estimate of $\underline{V}_d$, that is:

$$[\underline{V}^{(1)}{}_\infty - (\pi^\circ/\pi)] = \underline{V}_d\ [1 - (\pi^\circ/\pi)] \qquad [4.48]$$

A plot of $Z = [\underline{V}^{(1)}{}_\infty - (\pi^\circ/\pi)]$ as a function of $X = [1 - (\pi^\circ/\pi)]$ should therefore give a straight line with *zero intercept* and slope $\underline{V}_d$. Note that the introduction of the ratio π°/π enables one to use data of experiments carried out at different temperatures since the implicit temperature dependence of the osmotic pressure vanishes in that case. Furthermore, as observed by Hempling (1973), since the equilibrium cell volume is not a function of temperature, a unique value of $\underline{V}_d$ should be found. Another advantage of the above form is that, since the intercept must be zero, it reduces by one the number of points on the X-scale necessary to perform the linear regression.

To determine the volume of the granulocytes after equilibration with hypo- and hypertonic osmotica, a commercial PBS solution (Gibco Laboratories, Grand Island, N.Y.) containing sodium chloride (which behaves like an impermeable solute) and corresponding to an osmotic ratio with respect to isotonic condition of $\pi/\pi^{\circ} = 10$, was diluted with distilled water to provide 4 incremental tonicities, namely: $\pi/\pi^{\circ} = 0.5$, 1, 3 and 5. It was assumed that the increment in osmoticum volume after injection of an aliquot of cellular suspension had no significant impact on the bath concentration. Trials for mixing with 1x PBS solution (289 ± 5 mOsm) were conducted as controls to check for isotonicity of the osmoticum (for which theoretically no volume change of the cells should occur, as can be seen in Figure 4.1). The test solutions were then prepared with respect to that reference, namely: 0.5x PBS, 3x PBS and 5x PBS.

Equilibrium volume averages corresponding to cells exposed to osmotic ratios of $\pi^{\circ}/\pi = 1/3$ and $1/5$ for bath temperatures varying from 5°C to 30°C (by steps of 5 C°) were then fitted to equation 4.48 using a multiple linear regression procedure (BMDP1R, BMDP Statistical Software, 1982) which allowed the intercept to be forced to go through zero. An unweighted regression was used due to the relative homogeneity of the estimated variances of the means. It was assumed that this homogeneity was not affected by transforming the original average volumes to the Z form used as dependent variable in equation 4.48. The data corresponding to hypotonic runs were not used in this analysis since, in most cases, the equilibrium state had not been reached by the cells at the end of the monitoring. Nevertheless, the observed transients were well in line with

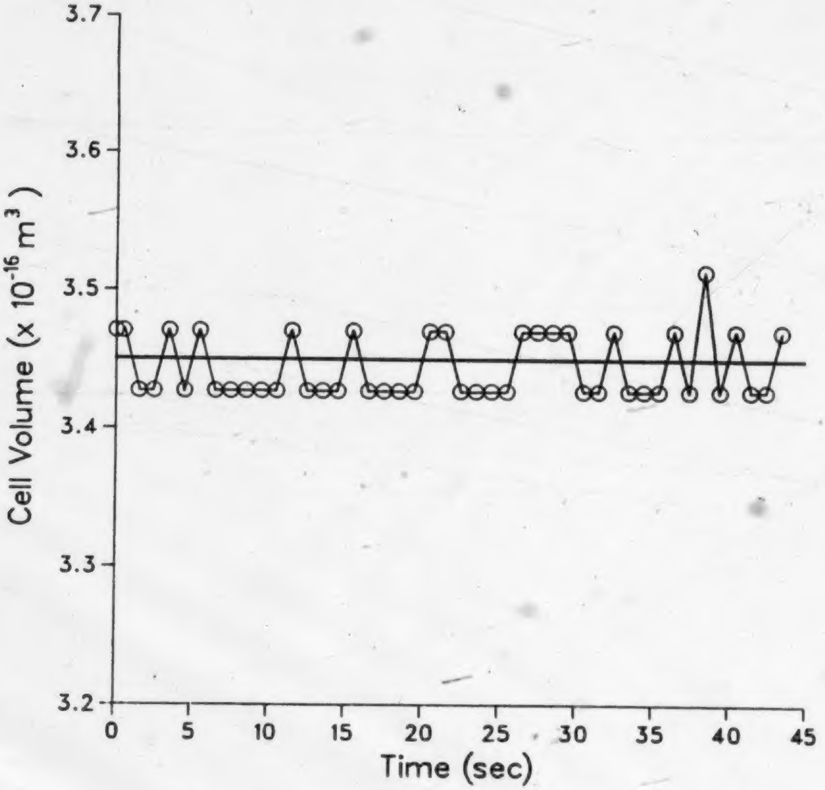

Figure 4.1 Measured average volumes of human granulocytes after exposure to an isotonic NaCl solution (1x PBS) at 20°C. Open circles, observed values; solid line, average.

similar volume changes measured by Diller and Bradley (1984) for human granulocytes exposed to equivalent stresses.

A *normal probability plot* and a *detrended normal probability plot* of the residuals obtained from the above analysis are shown in Figures 4.2 and 4.3. In the former plot the residuals, i.e. $Z_i - \hat{Z}_i$, where $\hat{Z}_i$ is the i-th *predicted* value, are plotted against the expected normal deviate corresponding to its rank. As anticipated, the strong straight-line relationship confirms that the data are from a *normal* distribution.

Figure 4.3 is similar to the normal probability plot except that the linear trend is removed. The vertical scale represents the differences between the expected normal values and the standardized values of the observations. That is, each observation, Z_i, is transformed into a standardized value, z_i, by subtracting the mean, $\bar{Z}$, and dividing by the estimated standard deviation, $\hat{\sigma}$, i.e.:

$$z_i = (Z_i - \bar{Z}) / \hat{\sigma} \qquad [4.49]$$

An even scatter of the differences around zero is an indication of *normality* for that particular plot. This result is especially crucial since it corroborates our assumption that the *average volumes* to be used in our study of the permeability coefficients respect the requirements inherent in least-squares analysis (see section 4.3.3).

An estimate of $\underline{V}_b$ of 0.425 ± 0.001 (*SDE*)[5] was obtained,

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[5] *SDE*: standard deviation of the estimated parameter.

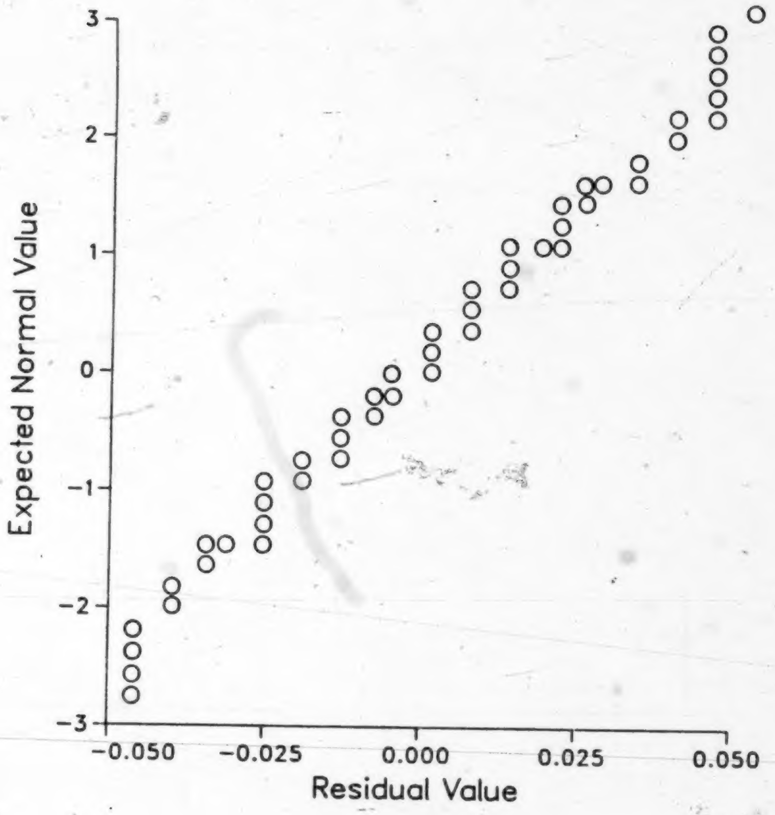

Figure 4.2 Normal probability plot of the residuals.

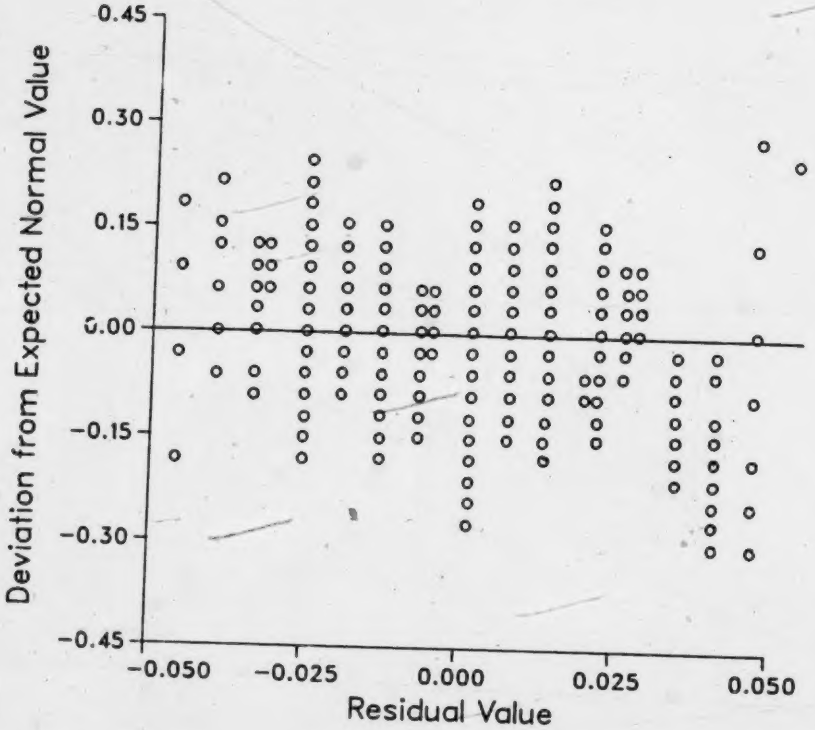

Figure 4.3 Detrended normal probability plot of the residuals.

which again compares very well with Hempling's finding (Hempling, 1973) of 0.42 ± 0.04 (*MEE*) for a similar population or with a value of 0.428 ± 0.005 (*SDM*) found by Savitz and coworkers (Savitz, Sidel and Solomon, 1964) for human red cells. Note that the increased precision in our case is due to employing 390 average volumes, corresponding to a sample of about 1.2×10^6 individuals.

The last physical parameter which must be evaluated in order to use equations 4.20 and 4.21 is the *cell membrane surface area*. For that matter, one could simply take as an estimate (assuming that granulocytes can be considered spheres) the surface area corresponding to the measured *isotonic* volume of the cells. In our case, though, the experiments performed in *hypotonic* media revealed that an important swelling of the cells was occurring, which was indicative that the above approach could not be used. It was therefore assumed, as discussed in section 4.1.1, that the membrane surface area would be the area corresponding to the *maximum* cellular volume achieved upon swelling. This turned out to be about 1.45-times the isotonic volume, i.e. a cell membrane surface area of approximately 3.05×10^{-10} m^2.

Let us note that the uncertainties attached to the values of $V^{(1)}_0$, A_0 and $\underline{V}_d$ do not manifest themselves in the determination of the macroscopic permeability coefficients since, as discussed in section 4.3.1, we assume that these coefficients are *not correlated* with the geometrical characteristics of the studied cells. Hence, we have taken these parameters to have *single* values rather than a distribution with variation about a mean. The reader interested in the influence of the statistical distribution of cellular properties on transport measurements can consult the work of Saari and Beck (1974) for more details. Let us just mention that, although the inclusion of such variabilities in the present analysis would have certainly improved the estimation of the probability density of the permeability coefficients, it represented a tremendous task that was not attempted.

5. SINGLE CELL BEHAVIOR: RESULTS AND DISCUSSION

5.1 General

When living cells are transferred from an isotonic solution to an osmoticum which is either *hypotonic* or *hypertonic*, the response of the cells will depend on the permeability of the plasma membrane to *water* and to the *solute* added to prepare the bathing solution. This gives rise to four types of situations, as illustrated in Figure 5.1:

1. The membrane is *impermeable* to the solute and the bathing solution is *hypertonic*. The cell will *shrink* to an equilibrium volume proportional to the concentration of the solute. The rate of shrinkage depends exclusively on the water permeability of the membrane.
2. The membrane is *impermeable* to the solute and the osmoticum is *hypotonic*. The cell will *swell* to an equilibrium volume if the physical and mechanical properties of the membrane can sustain the influx of water (this is usually achieved when the chemical potential of the bath is not too far from isotonicity). For animal cells and plant cells (the cell walls of which have been removed), lysis or irreversible changes to the membrane can occur if the stress is too great.
3. The membrane is *permeable* to the solute (but more permeable to water) and the osmoticum is *hypertonic*. The cell volume will transiently decrease, then increase to

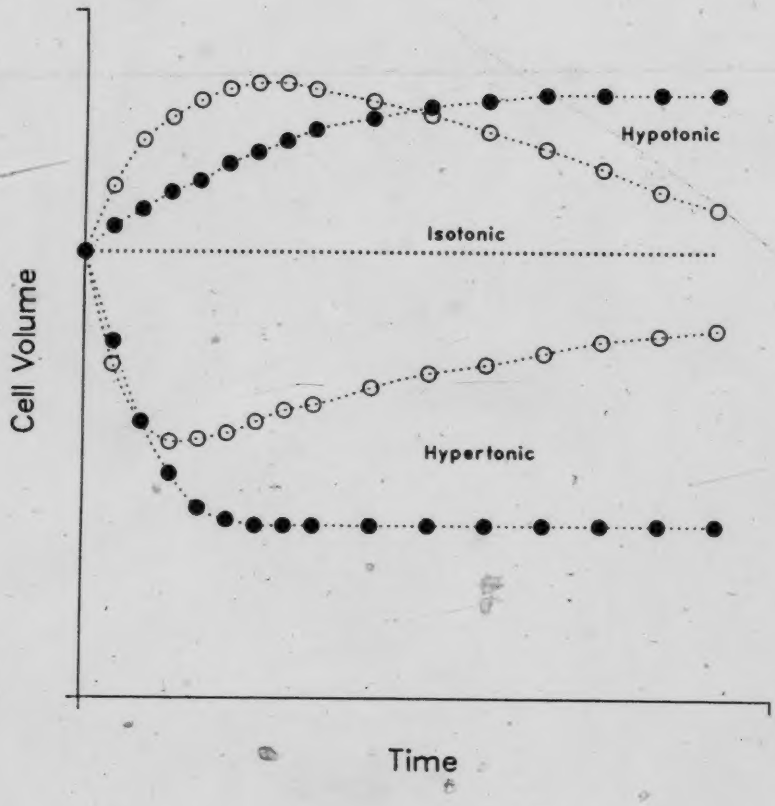

Figure 5.1 Hypothetical cell exposed to hypo- and hypertonic osmoticae, in the presence of a permeable and an impermeable solute. Open circles, permeable solute; closed circles, impermeable solute.

approximately its isotonic value. Initially, the cell shrinks in response to the increased extracellular chemical potential due to the permeant solute, until a dynamic equilibrium in intra- and extracellular potentials is reached. Meanwhile, with solute influx, water returns into the cell to maintain osmotic equilibrium. The rates of water and solute movement across the plasma membrane depend on the respective permeability coefficients of the species present, as well as on their *cross-coefficient*, which describes their interaction during transmembrane transport.

4. Same condition as in 3, but the extracellular solution is *hypotonic*. The transient behavior of the cell is more or less the reverse of the previous case. Initially, the cell swells in response to the decreased extracellular potential. Water *and* solute enter rapidly into the cell, then the cell volume decreases toward its isotonic value due to a relatively slow efflux of the solute and water.

In the present work, human granulocytes were exposed to environments corresponding to the first three cases. Estimates of their membrane macroscopic permeability coefficients were computed by fitting the observed transients to the model equations (described in section 4.1.2 and Chapter 3) using a *Modified Simplex method* implemented to allow for the calculation of the parameter standard errors. The reader will find the complete description of the algorithm in Appendix 1. The experiments were designed not only to allow for concentration dependence studies of the permeability coefficients but also to investigate a possible temperature sensitivity of the parameters. The assumptions which have gone into the analyses were disclosed previously, but will be briefly outlined here:

1. Ideality and diluteness of solutions. Departures from this assumption are supposed to be handled by the introduction of apparent concentrations (see section 4.1.1) and/or the use of the concept of apparent cellular nonosmotic volume (see section 4.4).

2. Constant solute(s) concentration(s) of the osmotica.

3. Cellular and apparent nonosmotic volumes do not change with temperature[1]. The latter remains constant during the osmotic transients.

4. No hydrostatic pressure gradient exists across the membrane.

5. Constant membrane area, chosen to correspond to the surface area of a sphere having the maximum volume attained by the cells upon swelling.

6. Independence of the permeability coefficients from the geometrical properties of the cells. This supposes that, if a dependence exists, it is lumped into the estimates obtained for the permeabilities (as is the case for any deviation from the above assumptions).

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[1] The temperature independence of $\underline{V}_d$ can be justified by referring to its "physical" definition (see section 4.4). At the hypothetical external infinite osmotic pressure, the material remaining in the cell is practically the *dry matter*, the density of which is known to be influenced very little by temperature variations.

5.2 Macroscopic permeability coefficients of human granulocytes

5.2.1 Studies with an impermeable solute (cases 1 and 2)

As described in section 4.4, a 10x PBS stock solution was used to prepare one hypotonic and two hypertonic media, that is: 0.5x, 3x and 5x PBS. Runs were performed at 6 different temperatures, namely: 5, 10, 15, 20, 25 and 30°C. In each case the osmoticum was placed in the counter chamber and was allowed to come to a new equilibrium temperature before injecting the cell suspension (which had been previously equilibrated to the same temperature). Complete mixing of the suspension was achieved in approximately 0.5 sec, which was a sufficiently short period to ensure good measurements in the initial phase of volume changes. On the average, three replicate runs were performed at each concentration-temperature combination, which permitted the estimation of the variances of the experimental data and the use of the weighted least-squares procedure described in section 4.3.3.

In the case of an impermeable solute, the system can be fully described by a unique parameter, the macroscopic phenomenological coefficient related to the solvent, L_{11}, since the phenomenological coefficient related to the solute and the associated cross-coefficient vanish (see section 2.2.3). However, as discussed previously, the inclusion of a temperature dependence in the description of the membrane permeability introduces a second parameter to be optimized, the apparent transfer energy, A_{ww}, along with the macroscopic permeability coefficient $\mathcal{P}^{\circ}_{ww}$, that is:

$$L_{ww} = \mathcal{P}^{\circ}_{ww}\ \bar{c}_{w}\ \exp[(A_{ww}/\mathcal{R})(1/T^{\circ} - 1/T)] \qquad [5.1]$$

where $\bar{c}_{w}$, the average concentration of the *solvent* across the barrier, is assumed to be well approximated by the inverse of its partial molar volume, $\bar{v}_{w}$, i.e.:

$$\bar{c}_{w} \cong 1 / \bar{v}_{w} \qquad [5.2]$$

Relation 5.2 is important since it is assumed *explicitly* that the permeability of the membrane to water is *independent* of *medium osmolality*. This supposition is a direct outcome of the approximation described by equation 3.2 and the ensuing choice of a *symmetric* reference for the concentration. Indeed, since we assume the system solutions to be dilute, it is reasonable to expect that the permeability of water will be unaffected by the presence of other species, especially in view that the solvent is omnipresent in the system and is far more "concentrated" than the other components. This statement is further supported by the fact that there is still no clear consensus as to the existence of such dependence for L_{ww} or as to the form it would take (Diller and Bradley, 1984).

A major outcome of the above approximation is that it permits direct comparison of the macroscopic permeability coefficient, $\mathcal{P}^{\circ}_{ww}$, with published values expressed in terms of the hydraulic permeability, *Lp*, since, as discussed previously, L_{1x} and L_{xx} are zero, and L_{11} is independent of concentration. Hence, referring to equation 2.22, *Lp* is given by:

$$Lp = \tilde{v}_1^2 L_{11} \qquad [5.3]$$

which becomes, after inserting equations 5.1 and 5.2 in relation 5.3:

$$Lp = \tilde{v}_1 \text{Þ}_1^\circ \exp[(A_{11}/R)(1/T^\circ - 1/T)] \qquad [5.4]$$

When human granulocytes were introduced into a hypertonic solution of sodium chloride, the expected progressive decrease in cell volume was obtained, as shown in Figures 5.2 and 5.3. These figures also demonstrate the influence of temperature on the osmotic response of the cells, with the longer time requirement for equilibration at lower temperatures. Each curve corresponds to an experimental trial selected as being representative of the replicate runs for that particular concentration-temperature combination. Each point on these transient lines represents the average volume of approximately 3,000 cells. Figure 5.4 pictures the behavior of cells put into the hypotonic osmoticum (0.5x PBS). The influence of temperature on the response of the cells is again observed with slower response at lower temperatures.

The complete sets of experimental data for the hypo-

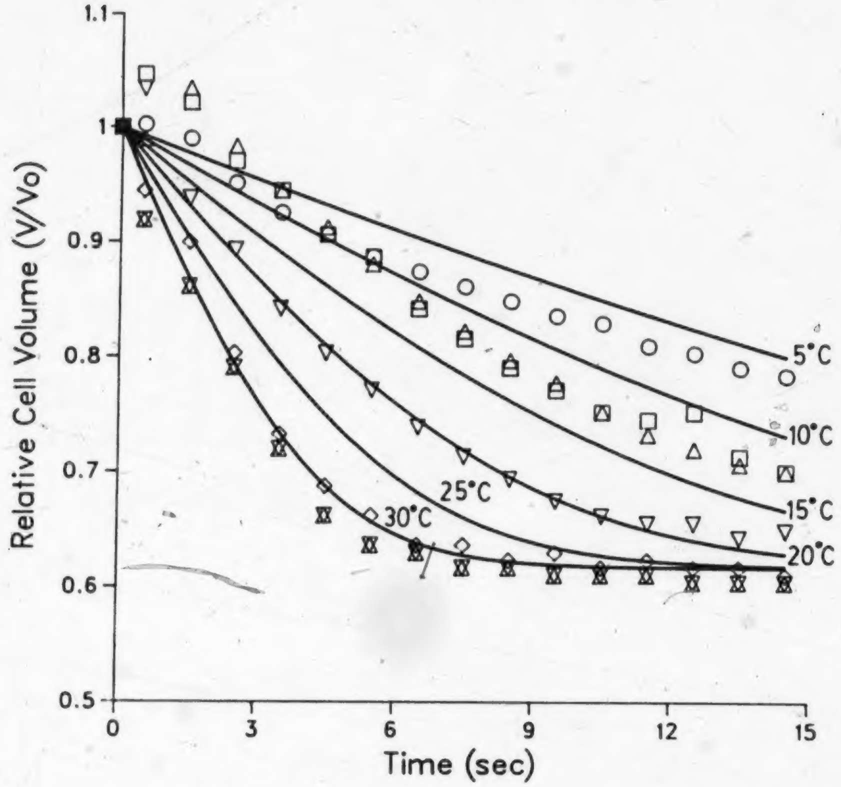

Figure 5.2 Transient volumes of human granulocytes after exposure to a hypertonic NaCl solution (3x PBS) at different temperatures. Solid lines, least-squares fit; symbols, observed volumes: ○ 5°C, △ 10°C, □ 15°C, ▽ 20°C, ◇ 25°C, ⧗ 30°C.

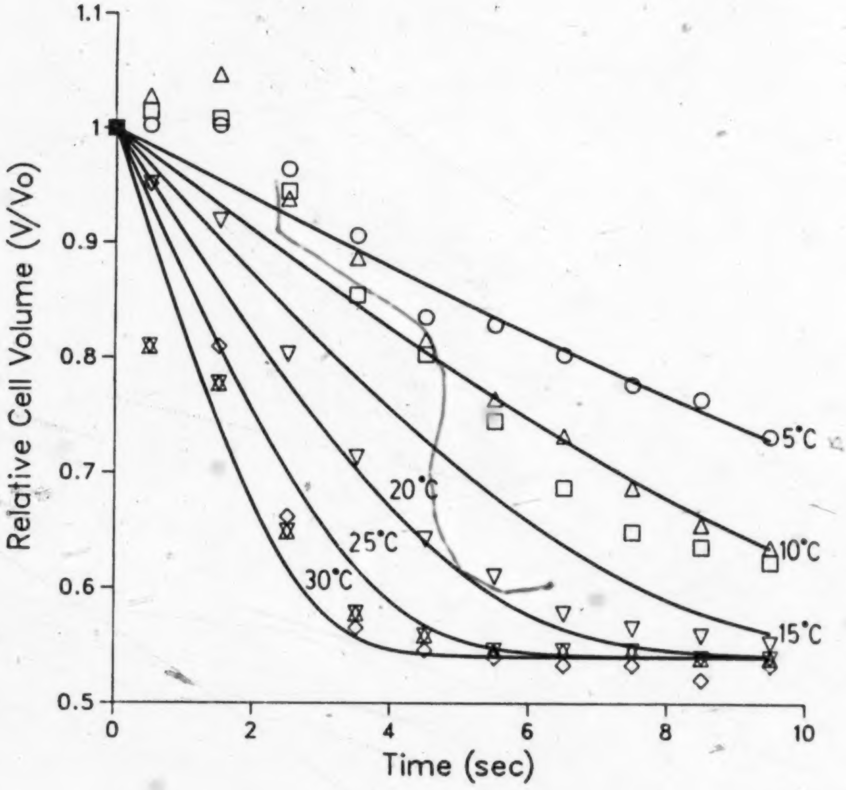

Figure 5.3 Transient volumes of human granulocytes after exposure to a hypertonic NaCl solution (5x PBS) at different temperatures. Solid lines, least-squares fit; symbols, observed volumes: ○ 5°C, △ 10°C, □ 15°C, ▽ 20°C, ◇ 25°C, ⧖ 30°C.

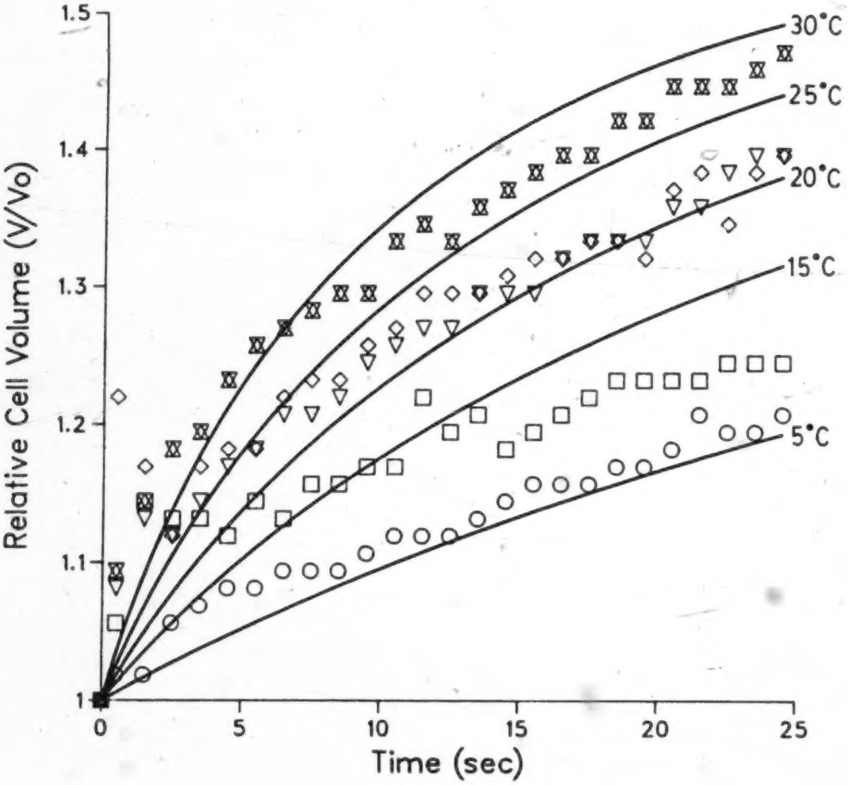

Figure 5.4 Transient volumes of human granulocytes after exposure to a hypotonic NaCl solution (0.5x PBS) at different temperatures. Solid lines, least-squares fit; symbols, observed volumes: ○ 5°C, □ 15°C, ▽ 20°C, ◇ 25°C, ⊠ 30°C.

and hypertonic runs[7] were fitted to the model equations (equations 4.20-4.25) along with equation 5.1 and the initial and boundary conditions described in section 4.1.2. Briefly, an integration routine (to be presented in Appendix 2) was repeatedly called by the optimization procedure (see Appendix 1) to generate predicted transients that were compared to the experimental data for *all the curves simultaneously* until a satisfactory fit was found (see Figures 5.2-5.4).

The discrepancies that can be observed in these figures between predicted and observed data are due, among other things, to the inevitable scatter of experimental observations and to the nature of the weighted least-squares procedure adopted in the analysis, which tends to anchor the solution to the data sets of better quality. This is particularly obvious for the 20°C runs where *6* replicates were available at each concentration level. It must be emphasized here that the *least-squares* curves presented in Figures 5.2-5.4 (and later on in Figures 5.5-5.7) are generated from *global* parameter values which have been found, in the *least-squares sense*, to most accurately describe *simultaneously* the behavior of the *entire set* of experimental curves under consideration. They are *not* equivalent to the least-squares curves that would have been obtained if the optimization procedure had been performed on each of them *individually*, which of course would have yielded better fits. However, considering, for example, that the predicted curves of Figures 5.2 and 5.3 correspond to transient volumes generated from the *same* set of permeability estimates (see Table 5.1) obtained by fitting *39* experimental runs at the same time, the agreement between observed and predicted volumes is more than satisfactory.

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[7] Due to incorrect adjustment during monitoring of the volume changes, the group of data corresponding to the hypotonic runs at 10°C was discarded.

The coefficients determined from the least-squares procedure are presented in Table 5.1 for both hypo- and hypertonic conditions. As can be seen, the values support the suggestion of Mazur (1981) that there is an exponential dependence of the water macroscopic phenomenological coefficient, L_{ww}, on temperature. The fitted curves result in a value of 4.76 ± 0.09 (*SDE*) x 10^{7} J kgmol^{-1} for the apparent transfer energy, A_{ww}, of the above macroscopic coefficient for *exosmotic flow* of water (outwards), whereas, for *endosmotic flow* (inwards), a value of 4.9 ± 0.1 (*SDE*) x 10^{7} J kgmol^{-1} is found. The corresponding macroscopic permeability coefficients (temperature independent form, P°_{ww}) are, respectively, 2.04 ± 0.02 (*SDE*) x 10^{-12} kgmol N^{-1} s^{-1} and 6.05 ± 0.07 (*SDE*) x 10^{-12} kgmol N^{-1} s^{-1} at the reference temperature, 20°C.

Our findings are in line with values of 7.7 ± 0.8 (*SDE*) x 10^{7} J kgmol^{-1} for A_{ww} and of 3.49 x 10^{-12} kgmol N^{-1} s^{-1} (*SDE* not available) for P°_{ww} at 20°C, reported by Hempling (1973) for exosmotic flow of water from human granulocytes. McGann and coworkers (McGann, Turner, Mannoni and Turc,

Table 5.1 Water macroscopic permeability coefficients and apparent transfer energies of human granulocytes exposed to impermeable NaCl at 20°C and 25°C

Direction of flow	Temperature of reference T° (°C)	Macroscopic permeability coefficient $Þ^{\circ}_{ij}$ (kgmol N^{-1} s^{-1})[1]	Apparent transfer energy A_{ij} (J $kgmol^{-1}$)[2]
Exosmotic	20	2.04 ± 0.02[3]	4.76 ± 0.09
		3.49 ± n/a[4]	7.7 ± 0.8[4]
		2.19 ± n/a[5]	n/a
	25	2.89 ± 0.05	4.96 ± 0.09
Endosmotic	20	6.05 ± 0.07	4.9 ± 0.1
	25	8.48 ± n/a	n/a

[1] The coefficients should be multiplied by 10^{-12} to give them their actual numerical magnitudes.

[2] The coefficients should be multiplied by 10^{7} to give them their actual numerical magnitudes.

[3] Standard deviation of the estimate.

[4] Hempling (1973)

[5] McGann *et al.* (1983); all other cases this study.

1983) obtained a value of 2.19 x 10^{-12} kgmol N^{-1} s^{-1} (*SDE* not available) for Þ°_{i}, at 20°C for human granulocytes subjected to similar stresses. This result compares very well with our estimate, which tends to support the above authors' suggestion that the higher value reported by Hempling may be related to alterations in permeability characteristics of the cell membrane caused by the hypo-osmotic stress used to lyse the erythrocytes in the leukocyte suspension, or to contributions from contaminating lymphocytes.

The most interesting finding, though, is the striking difference which exists between the estimates obtained from analyzing hypotonic versus hypertonic transients. As can be seen in Table 5.1, the water permeability is indeed 3-times higher for endosmotic flow than for exosmotic flow. This result seems to substantiate the idea that *rectification of osmotic flow* may occur in human granulocytes, i.e. that the permeability of the membrane to water is dependent upon the *direction of flow*, a phenomenon which was reported by Farmer and Macey (1970) for bovine and human red cells.

Rectification of water flow is well known in plant cells, but its cause remains obscure (Dainty, 1963). On the other hand, there are a lot of controversies as to the existence of that phenomenon in animal cells (House, 1974; Diller and Bradley, 1984). Some possible explanations have nevertheless been proposed to explain that peculiar property of the cell membrane.

Firstly, as discussed by Dainty (1963), the different rates of movement of water inwards and outwards across a membrane may simply be due to the fact that the limiting barrier becomes less hydrated when exposed to a lower external water chemical potential and, accordingly, becomes less permeable. It is important to understand that the movement of molecules across the membrane may rely in most part on the presence of *water* in the structure, since it is well known (Hartley, 1957) that in many polymers the diffusion coefficient of water is very strongly dependent on the degree of hydration of the polymer. A second possibility involves a dependence of membrane structure upon the direction of water flow; in particular, a valve-like action occurring within aqueous channels could lead to a direction-dependent value of Þ°_{s}, by changing the "effective pore radius" (Farmer and Macey, 1970). One further possibility may be ascribed to the "effective membrane area" available for transfer. The inward flow of water may tend to "unfold" the membrane, with the result that a maximal membrane area is accessible for transport of water into the cell.

In any event, until more information becomes available, we will have to confine our remarks to the view that considerable structural reorganization of the membrane must occur not only in response to the direction of flow but also to changes in temperature. Indeed, as noted by Hempling (1973), how can we explain the high apparent transfer energy, A_{ss}, under hypo- and hypertonic conditions (which seems to be related to the same changes since the values are not significantly different) when *unconstrained* movement of water across the membrane would lead to values ranging from 1.26 to 2.09 x 10^{7} J $kgmol^{-1}$?

5.2.2 Studies with a permeable solute (case 3)

Figures 5.5, 5.6 and 5.7 show the resulting changes in average cell volume when human granulocytes were exposed to solutions containing dimethyl sulfoxide (DMSO) at concentrations of 1, 2 and 3 molar, respectively. These osmotica were prepared by adding DMSO to isotonic PBS solution and the experimental procedure followed exactly the sequence described in section 5.2.1. The measurements were carried out at 3 different temperatures for each concentration level, namely: 20, 25 and 30°C.

Dimethyl sulfoxide (MW 78.3) is used widely as a *cryoprotective* compound in the long-term preservation of viable cells and tissues at low temperatures. As can be seen, the transient changes in cell volume shown in these figures indicate that the plasma membrane is *permeable* to DMSO. The sharp initial decrease in cell volume reveals, furthermore, that water moves across the barrier faster than does the solute.

As for Figures 5.2-5.4, the most representative curves were selected for the purpose of illustration along with the accompanying least-squares lines. The reader should note that in some instances the agreement between fitted and

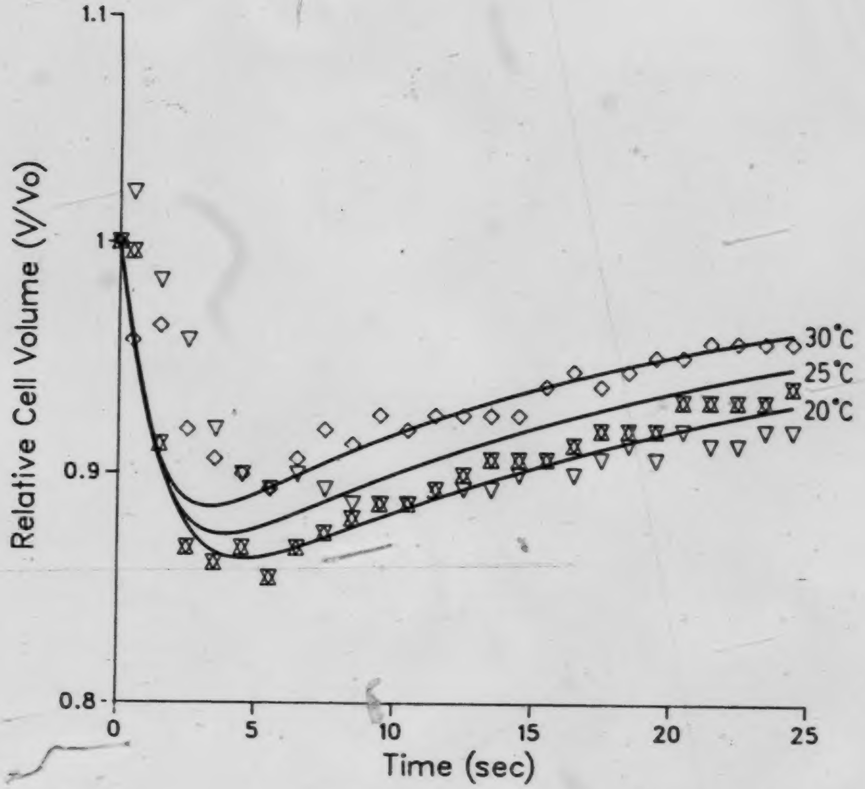

Figure 5.5 Transient volumes of human granulocytes after exposure to a hypertonic 1M DMSO solution at different temperatures. Solid lines, least-squares fit; symbols, observed volumes: ▽ 20°C, ◇ 25°C, ⧖ 30°C.

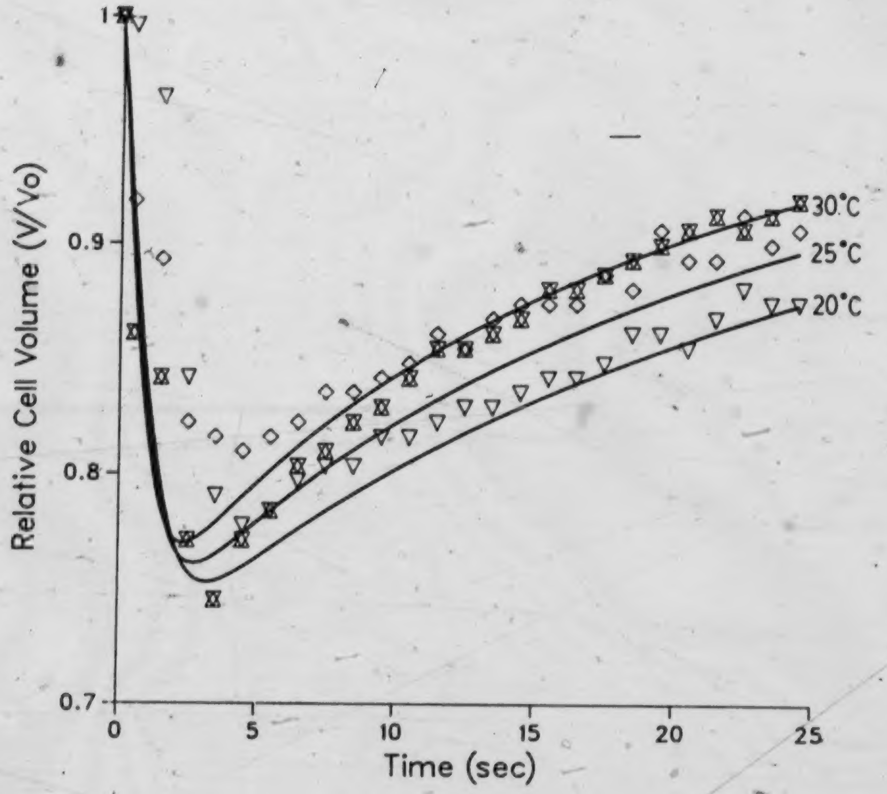

Figure 5.6 Transient volumes of human granulocytes after exposure to a hypertonic 2M DMSO solution at different temperatures. Solid lines, least-squares fit; symbols, observed volumes: ▽ 20°C, ◇ 25°C, ⧖ 30°C.

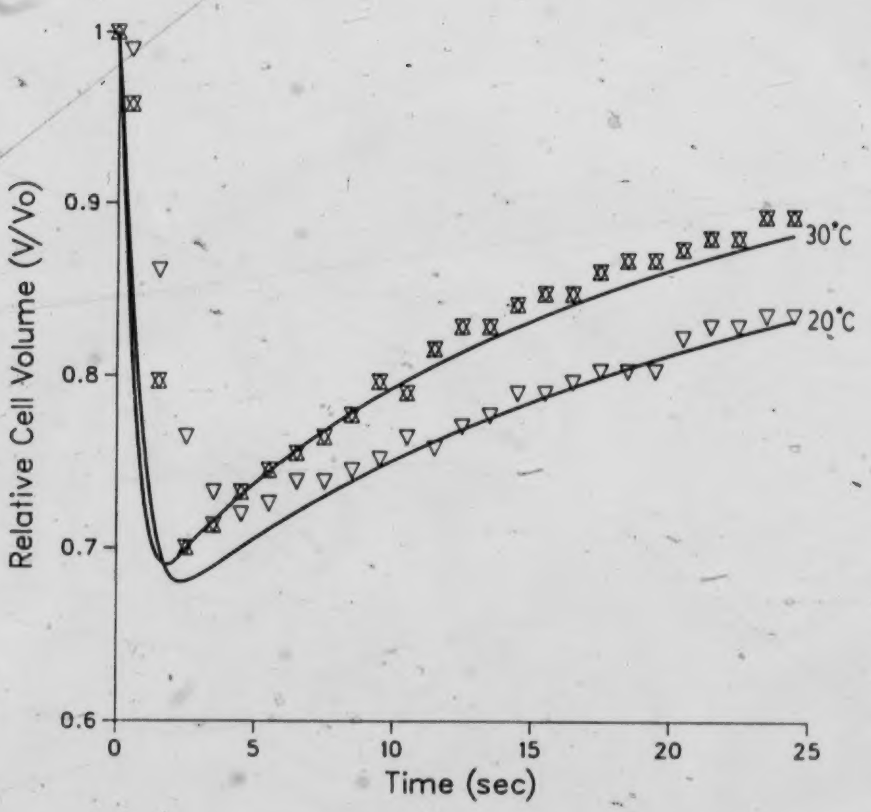

Figure 5.7 Transient volumes of human granulocytes after exposure to a hypertonic 3M DMSO solution at different temperatures. Solid lines, least-squares fit; symbols, observed volumes: ▽ 20°C, ⊠ 30°C.

observed data is poor (1M DMSO at 25 and 30°C, for example). This is attributable to the fact that, in contrast to the previous analysis, the data available for treatment are not extensive. Some concentration-temperature combinations are indeed described only by one run instead of the usual triplicates. It was therefore normal for the least-squares procedure to favor some combinations more than others, especially in view of the fact that all the available curves were fitted simultaneously. Let us point out however that the range in osmoticum composition and temperature covered by the experiments, even with these discrepancies, is more than sufficient to ensure proper statistical analysis and estimation of the permeability coefficients for that system.

The influence of temperature on the dynamic osmotic changes can be judged by observing the predicted transients in Figures 5.5-5.7. As can be seen, the magnitude of the volume change and the equilibration time increase as temperature decreases. The latter behavior has been disclosed previously for granulocytes exposed to an impermeable solute (see section 5.2.1), whereas the former pertains to the presence of a permeable solute in the system and its interaction with the diffusing solvent.

As can be seen in Table 5.2, there are now *6* coefficients describing the mass transfer across the plasma membrane: 3 macroscopic permeability coefficients $þ^{\circ}_{ss}$, $þ^{\circ}_{sx}$ and $þ^{\circ}_{xx}$, and 3 apparent transfer energies, A_{ss}, A_{sx} and A_{xx} (where the subscript s refers to the solvent, and x to the

Table 5.2 Macroscopic permeability coefficients and apparent transfer energies of human granulocytes exposed to permeable DMSO at 25°C

Nature of coefficient	Macroscopic permeability coefficient $\text{Þ}^{o}_{(\ )}$ (kgmol N^{-1} s^{-1})[1]	Apparent transfer energy $A_{(\ )}$ (J $kgmol^{-1}$)[2]
Straight (water)	4.6 ± 0.2[3]	2.6 ± 0.2
Cross (water-DMSO)	0.174 ± 0.008	5 ± 1
Straight (DMSO)	0.148 ± 0.002	2.9 ± 0.1

[1] The coefficients should be multiplied by 10^{-12} to give them their actual numerical magnitudes.

[2] The coefficients should be multiplied by 10^{7} to give them their actual numerical magnitudes.

[3] Standard deviation of the estimate.

solute). This is a direct outcome of the use of relations 3.9 and 3.10 to describe the temperature and concentration dependencies of the 3 macroscopic phenomenological coefficients, L_{ss}, L_{sx} and L_{xx}, required by the theory of irreversible thermodynamics to relate the fluxes of the two diffusing species (water and DMSO) to their conjugated and nonconjugated forces (see section 2.2.3). More explicitly, the following equations were used in the analysis:

$$L_{kk} = \text{Þ}^{\circ}_{kk}\ \bar{c}_k \exp[(\mathcal{A}_{kk}/\mathcal{R})(1/T^{\circ} - 1/T)]$$

$$k = \text{water (s) and DMSO (x)} \qquad [5.5]$$

and

$$L_{jk} = \text{Þ}^{\circ}_{jk}\ \surd(\bar{c}_j\ \bar{c}_k) \exp[(\mathcal{A}_{jk}/\mathcal{R})(1/T^{\circ} - 1/T)]$$

$$j,k = \text{water (s) and DMSO (k) and } j \neq k \qquad [5.6]$$

where $\bar{c}_j$ and $\bar{c}_k$ are, as before, the average concentration of the given species across the membrane. Note that, as for equation 5.1, the approximation 5.2 was used for $\bar{c}_s$. In the case of $\bar{c}_x$, the value of the mean concentration was computed, as required, as a *point function* by numerically integrating equation 4.21, which describes the variation of the intercellular concentration of the species. The reader can now understand more clearly the *local* nature of the L coefficients (see section 4.3.2) as opposed to the Þ° and $\mathcal{A}$ parameters, which describe the *entire* experimental range.

Turning back to the dynamic volume changes, it can be seen from the estimates given in Table 5.2 that the observed decrease in the magnitude of osmotic transients can be linked, among other things, to the existence of a strong *interaction* between the species. Indeed, referring to the discussion of section 2.2.4, the cross-coefficient indicates that there is *mutual drag* ($\text{Þ}^{\circ}_{sx}>0$) between water and DMSO when they cross the barrier. This implies that the outflow of water in the early stage of shrinkage will tend to slow down the entry of DMSO whereas in the later stage (on the other side of the minimum) the continuous inflow of DMSO will accelerate the entry of water back into the cell to compensate for the osmotic imbalance. Let us note that by virtue of the symmetry of the cross-coefficient the reverse is also true with DMSO hindering the exit of water and water "pulling" in DMSO. It is therefore obvious that the magnitude of this interaction will control to a certain extent the dynamics of volume changes over and above the intrinsic permeabilities of the species (straight-coefficients).

For the purpose of illustration, let us take the values presented in Table 5.3 for the three permeability coefficients, Þ_{ss}, Þ_{sx} and Þ_{xx}, as a function of the 3 experimental temperatures, 20, 25 and 30°C. These parameter values were computed from the optimized Þ°'s and A's at the reference temperature, 25°C.

As can be seen, due to its high apparent transfer energy, A_{sx} (see Table 5.2), the interaction parameter, Þ_{sx}, increases substantially with temperature in the studied experimental range (≃98%). The same observation applies for

Table 5.3 Effect of temperature on the macroscopic permeability coefficients

Temperature (°C)	Macroscopic permeability coefficient[1] $\bar{P}_{(\ )}$ (kgmol N^{-1} s^{-1})		
	(water)	(water-DMSO)	(DMSO)
20	3.9	0.123	0.121
25	4.6	0.174	0.148
30	5.5	0.243	0.179

[1] The coefficients should be multiplied by 10^{-12} to give them their actual numerical magnitudes.

$Þ_{ss}$ and $Þ_{xx}$, although to a lesser extent (≈41% and 48%, respectively). This indicates that the mass transfer phenomena are accelerated as temperature increases, with the result that the periods of net outward and inward volume flow are shortened. This is particularly visible in the swelling parts of the curves presented in Figures 5.5-5.7. The position of the minimum (no net volume flow), the magnitude of which decreases as temperature increases, is attributable to the interaction term which becomes predominant relative to the corresponding incremented values of the straight permeabilities as temperature is augmented. This tends to accentuate the role of the nonconjugated forces in the definition of the fluxes (see equation 4.22) and accordingly alters the location of the minimum. It is interesting to note that, according to the same dependencies, a continuous *decrease* in temperature could practically lead to *uncoupling* of the fluxes since the value of the interaction term diminishes much faster than the values of the straight-coefficients for the same temperature decrements.

A point which is worth mentioning in relation to the water-DMSO interaction is the fact that the estimate of $Þ^{\circ}_{ss}$ at 25°C (4.6 ± 0.2 [*SDE*] x 10^{-12} kgmol N^{-1} s^{-1}) lies approximately half-way between the water permeabilities obtained at 25°C (see Table 5.1) for the case study with an impermeable solute, for both exosmotic and endosmotic flow, namely: 2.89 x 10^{-12} kgmol N^{-1} s^{-1} and 8.48 x 10^{-12} kgmol

N^{-1} s^{-1}, respectively (the latter value was calculated from the coefficients obtained at 20°C using equation 5.1). If we consider that most of the osmotic transients in DMSO studies are related to endosmotic flow, this non-negligible decrease in Þ°_{ss} must be due to the presence of the solute. If one assumes that DMSO has no effect on the properties of the membrane, then this decrease must be attributable to the mutual drag between the diffusing species in both directions, which tends to "average" the value of the overall permeability. It seems however that this phenomenon could be linked to some changes, induced by the presence of DMSO, in the structural properties of the membrane and/or its effect on the intracellular state of water. This hypothesis appears to be substantiated by the recent findings of Rule *et al.* (1980) and Hempling and White (1984) who reported a reduction in water permeability by 50% for cells which were allowed to equilibrate with a 0.6M DMSO solution *before* being exposed to a hyperosmotic NaCl osmoticum, as compared to control cells subjected directly to the hyperosmotic stress.

Comparison with published estimates is difficult for two reasons. Firstly, extensive studies on the temperature and concentration dependencies of macroscopic permeability coefficients in systems containing a permeable solute are scarce. Secondly, most researchers quote their values in terms of the "practical" permeability coefficients introduced in section 2.2.4, namely: the hydraulic conductivity, *Lp*, the reflection coefficient, σ, and the solute permeability, ω. It is therefore necessary to transform one set of coefficients in terms of the other if one wishes to discuss possible analogies between the findings. This was done in our case by employing equations 2.22, 2.23 and 2.24, which allow calculation of the practical parameters from known values of the macroscopic phenomenological coefficients (L form). The problem with such transformation, though, is that it leads to *local* values of *Lp*, σ and ω (since, as discussed previously, the L coefficients are a function of point values of concentrations) which have to be compared to integrated parameters that describe the entire experimental range. Values of the coefficients were then computed for 2 limiting situations: the initial conditions which correspond to the minimum values that $\bar{C}_x$ can take, and hypothetical asymptotic average concentrations corresponding to the values that $\bar{C}_x$ would take if both internal and external solute concentrations were the same. This permitted coverage of the widest possible range of practical parameter values. The calculations were performed at the reference temperature of 25°C for a 1M DMSO osmoticum and are reported in Table 5.4. Note that we have not attempted to compute the errors on these quoted values.

Our results with the human granulocytes may be compared qualitatively to coefficients obtained by Hempling and White (1984) in their study on the permeability of cultured

Table 5.4 Calculated practical permeabilities from estimates of the macroscopic permeability coefficients at 25°C

	Hydraulic conductivity Lp (μm min^{-1} atm^{-1})	Reflection coefficient σ (dimensionless)	Solute permeability ωRT (μm min^{-1})
This study[1]	0.52 ↔ 0.53	0.47 ↔ 0.58	21.12 ↔ 21.71
Hempling and White (1984)[2]	0.46	0.65	9.3

[1] Human granulocytes.
[2] Cultured megakaryocytopoietic cells of the rat.

megakaryocytopoietic cells of the rat to DMSO, since estimates with human granulocytes are not available. In their work, Hempling and White exposed cultured cells to 0.6M DMSO solution at 25°C and found values for the hydraulic conductivity, *Lp*, the permeability of the membrane to DMSO, ωRT, and the reflection coefficient, σ, of 0.46 $\mu m\ min^{-1}\ atm^{-1}$, 9.3 $\mu m\ min^{-1}$ and 0.65, respectively. As can be seen from Table 5.4, these estimates agree quite well with our findings, at least in order of magnitude, with the values of the hydraulic conductivity being exceptionally close if we consider that these estimates come from two different parent populations. Furthermore, the behavior of the reflection coefficients with respect to the solute permeabilities concur with the expectation from the well-spread porous membrane theory that, when σ falls, ω must increase (see Villars and Benedek, 1974).

The temperature variation of the practical coefficients is illustrated in Table 5.5. These values were computed from the apparent transfer energies and the macroscopic permeability coefficients listed in Table 5.2 using the initial conditions to be encountered in a 1M DMSO solution (lower limit). As expected, the water permeability, *Lp*, and the solute permeability, ωRT, increase with temperature. Accordingly, the reflection coefficient decreases, which is again in agreement with the porous membrane theory. It is interesting to note that Hempling (1967) found the same behavior for *Lp* and ωRT in his study of Ehrlich mouse

Table 5.5 Effect of temperature on the practical permeability coefficients

Temperature (°C)	Hydraulic conductivity *Lp* (μm min^{-1} atm^{-1})	Reflection coefficient σ (dimensionless)	Solute permeability ωRT (μm min^{-1})
20	0.43	0.54	17.25
25	0.52	0.47	21.12
30	0.62	0.41	25.55

ascites tumor cells, but failed to show any temperature sensitivity for σ. As discussed in section 2.2.4, this could be partly due to the fact that this author fitted simultaneously *correlated* coefficients (ω and σ) with the result that the temperature variation of σ might have been lost. Furthermore, a close look at the optimization procedure adopted by Hempling for curve fitting shows that the author carried out a "*single-factor-at-a-time*" search, which is known on some occasions to converge on misleading optima (Morgan and Deming, 1974).

6. CELL TO TISSUE BEHAVIOR: PRELIMINARIES

We have tried in the preceding chapters to expose some important facts on the application of the theory of irreversible thermodynamics to the study of biological systems, particularly, in the characterization of cell membrane properties in view of the estimation of membrane permeability coefficients from cell volume change data. The theory of irreversible thermodynamics proves to be a powerful tool to investigate irreversible processes which are commonly observed and studied in biological sciences. Its phenomenological nature provides a notation and a format which are appealing since they lead to a directly usable knowledge of the interactions involved in the system under study.

The preceding discussion should also emphasize the necessity to respect the requirements of the statistical theory if one wishes to make valid inferences from experimental observations, a point which, as mentioned earlier, is often overlooked by most researchers.

To our knowledge, this is the first time that such a fundamental approach has been applied in the context of osmometric studies in an attempt to reconcile the description of transport phenomena across biological membranes with a notation which is *intrinsically structure-independent*. It is interesting to see that relatively simple temperature and concentration dependencies (equations 3.9 and 3.10) permit a rather accurate characterization of the macroscopic phenomenological coefficients describing the permeability properties of a given population of cells. The optimized values of the phenomenological parameters offer the advantage of allowing not only comparison of the relative size of the coefficients but also to test the importance of each component flux in the overall cell response. Simplifying assumptions based on these analyses are then justified since they rely on quantitative ground.

With the development of a formal approach to the description of the cell behavior, it becomes possible to extend our study to the description of the behavior of an *arrangement* of such entities, the *tissue*. The analysis consists in casting the cellular units in a structured environment and in describing how the support matrix affects the overall mass transport phenomena over and above the influence of each cell composing the medium. In view of the type of tissues to be considered in the following chapters, it is important to point out here that the preceding theoretical considerations, although demonstrated with animal cells, can be extended without loss of generality to plant cells.

The next sections will be devoted to presenting how the theoretical formalism developed for describing and characterizing osmotically induced mass transfer in single cell suspensions can be used to depict similar processes occurring in complex cellular structures. The purpose of the second part of the study is to expose the theoretical approach used to approximate mass transport phenomena in a tissue environment, more precisely, in structures such as storage tissues of higher plants, namely, carrot, beet or potato roots, and to present a mathematical model developed from it[*].

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[*] It is important to emphasize that the present study is concerned with the description of mass transport processes in *living systems*, i.e. systems subjected to treatments that preserve their structural integrity. The dehydration phenomena to be considered remain well within the region of *osmotic systems*.

7. GENERAL ASPECTS OF ISOTHERMAL MASS TRANSFER IN COMPLEX CELLULAR STRUCTURES: A REVIEW

7.1 Mass transfer in porous media

7.1.1 General

Numerous studies have been done in chemical or petroleum engineering on mass transfer in porous media. Models exist, but are generally too specific to be applied directly for describing mass transport phenomena in biological materials. As we can imagine, the main restriction resides in the intrinsic nature of biological tissues, which hardly compare with the systems encountered in these fields (Rotstein and Cornish, 1978a).

Biological tissues are mostly porous or cellular substances in which a gas and/or a liquid are immobilized in a solid matrix. The rate of mass transfer by *molecular diffusion* in such structures can normally be predicted by appropriate solutions of the generalized second order Fick equation (Loncin, 1979), that is, for the i-th species of an m multicomponent system:

$$\partial c_i/\partial\theta = \mathrm{div}(\Sigma_k \eth_{ki} \cdot \mathrm{grad}\ c_k)$$
$$k = 1,2,\ldots,m-1 \qquad [7.1]$$

where $c_{i(k)}$ is the molar concentration of the i(k)-th species, θ is the time variable and $\eth_{ki}$ is a *generalized multicomponent* diffusion coefficient for the pair k-i not identifiable in general with *binary* diffusion coefficients (see Miller, 1960; Chandrasekaran and King, 1972; Clazie, Klein and Vermeulen, 1967)[9].

Biological systems however have, for the most part, a strong *anisotropic*[10] structure and, although exact analytical or numerical solutions of equation 7.1 are mathematically attainable, it is in general impossible due to a lack of information on the variations of the diffusivity of the diffusing substances in the various parts of these multilayer cellular structures. That is why it is normally assumed that the transfer is *unidimensional* (in some cases it is reasonable to suppose that changes along one direction may not alter substantially the order of magnitude of the coefficient), and to support this assumption one uses an *effective* or *apparent diffusivity*, $d_{ki}(eff)$, which can be determined experimentally (Biswal and Le Maguer, 1984), such that equation 7.1 becomes:

$$\partial c_i/\partial \theta = \partial/\partial z\ [\Sigma_k d_{ki}(eff) \cdot \partial c_k/\partial z] \qquad k = 1,2,\ldots,m-1 \qquad [7.2]$$

where z is the direction of diffusion.

For low concentrations of component i, the cross-diffusion coefficient, $d_{ki}(eff)$, $i \neq k$, tends to zero

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[9] We refer the reader to the List of Symbols for a complete description of the notation used in the following chapters.

[10] A material is called *anisotropic* when its properties, and in particular the diffusion of mass and/or heat, are dependent on the *direction* considered, as opposed to an *isotropic* material, where the properties are not a function of the direction considered (Loncin and Merson, 1979).

(Bird, Stewart and Lightfoot, 1960), i.e. the interaction of component k on the diffusion of component i is negligible. Hence, relation 7.2 can be further simplified to lead to:

$$\partial c_i/\partial\theta = \partial/\partial z\ (Đ_i \cdot \partial c_i/\partial z) \qquad [7.3]$$

where $Đ_i$ is the *apparent pseudo-binary diffusivity* of species i in the cellular structure, which again can be determined experimentally (Loncin, 1979; Shirazi and Riley, 1981).

Analytical solutions of equation 7.3 are available for idealized geometries (Crank, 1975) which allow the prediction of the concentration of the diffusing substance as a function of time and distance in the tissue, provided though that $Đ_i$ is known. For example, for the special case of diffusion in a solid that can be assimilated to a *semi-infinite* body, a solution of equation 7.3 is given by:

$$c_i(z,\theta)/c_i{*} = \mathrm{erfc}\{z\ /\ [2\ \sqrt{(Đ_i\ \theta)}]\} \qquad [7.4]$$

where $c_i(z,\theta)$ is the concentration of species i at a distance z and at time θ. Note that this solution is valid if the *initial* concentration $c_i(z,0)$ is equal to zero through all the solid material except for $z = 0$ (surface) where it is constant and equal to $c_i{*}$. It is assumed, furthermore, that $c_i{*}$ is in equilibrium with a given surrounding of *constant* composition. As can be seen, equation 7.4 can be solved for $Đ_i$ and can therefore be used to estimate the apparent diffusivity from experimental data

(Crank, 1975).

Although it is clear that at the *microscopic* level the diffusion process is *multidimensional* and therefore is inconsistent with the above assumption, the use of apparent diffusivities, $\text{đ}_{ki}(\text{eff})$ or Đ_{i}, is justified on the basis that the *macroscopic* movement of the solutes in the tissue can be *globally* considered unidimensional (Nicolas and Duquenoy, 1974). It is obvious nevertheless that the approximation of a multidimensional phenomenon by a unidimensional solution using an effective or apparent coefficient is the consequence of a lack of sufficient knowledge of the diffusion mechanisms in cellular tissues. Therefore, in an attempt to shed some light and explain to a certain extent the nature of this apparent diffusivity, we tried to derive a model that would describe this "*real life*" diffusion from the knowledge of the diffusivity of the diffusing substances in dilute solutions, which can be predicted with reasonable accuracy; the geometrical characteristics of the biological structure of interest; the possible interactions of the diffusing substances with the cell walls and membranes; and, finally, the exchanges that could occur with the intracellular material itself.

7.1.2 The obstacle approach

The most common and accepted method of simulating and/or predicting the apparent or *bulk* diffusion in a porous solid can be called the *obstacle approach*. Basically, the diffusion of a substance in the studied medium is believed to be hindered by the presence of obstacles like fat globules, fibers, cells, etc. The presence of these obstacles causes therefore a slowing down in the overall diffusion process (Zonji, 1969) which can be easily visualized by comparing the lower experimental apparent diffusivity, Đ_i, to the diffusivity of the that species in *solution*, D_i, i.e. the diffusivity of the diffusing substance in the *absence* of a porous medium.

Under the *implicit* restriction of *bulk unidirectional* diffusion in a *homogeneous*'', *isotropic* macroporous structure, many empirical correlations or model equations based on factors that *quantify* the complexity of the medium have been proposed to predict the effective or apparent diffusivity from the value of the "unhindered" coefficient (Van Brakel and Heertjes, 1974). The general approach is to try to relate Đ_i to D_i through a *correction factor*, ψ, referred to as the *diffusibility* of the porous structure, that is:

$$\text{Đ}_i = \psi D_i \; ; \; 0 \le \psi \le 1 \qquad [7.5]$$

The diffusibility is usually a function of physical characteristics of the medium, such as the *porosity* or *void fraction*, ϵ, defined as the fraction of the total volume of

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''*Homogeneity* in this context characterizes a medium which shows a relative coherence and repetition in its structural elements, whereas *heterogeneity* implies that the structural elements of the medium are different in nature and that the overall structure lacks unity.

the matrix which is *effectively* available for diffusion, and the *tortuosity*, τ, which is defined as the ratio of the real distance that a molecule has to travel in the medium as part of a *macroscopic unidirectional* flow in comparison to the distance covered *normal* to the surface (Geankoplis, 1978). These factors can be either measured experimentally or predicted from pore space models (Van Brakel and Heertjes, 1974).

Let us take, for example, the following empirical relation linking ψ to ϵ and τ (Geankoplis, 1978):

$$\psi = \epsilon / \tau \qquad [7.6]$$

As can be seen, if one has access to estimates of these physical factors and if relation 7.6 is appropriate, it is then theoretically possible, if we further assume that the slowing down of the diffusion process is purely due to *physical interferences*, to predict the apparent diffusivity, $Đ_i$ (through equations 7.6 and 7.5), which in turn can be used in equation 7.4 (or similar ones) to simulate the mass transfer of a given component in the structure of interest.

Besides the fact that this method seems suitable to a certain extent to approximate diffusion in *inert* systems like cheeses (Geurts, Walstra and Mulder, 1974) or gels (Nicolas and Duquenoy, 1974), studies on *living* systems such as beet or carrot roots, suggest that this approach is quite satisfactory only in the presence of *dead* or *badly damaged* cells in the matrix, i.e. for systems that become *non-osmotic*. It appears in that case that the cells are unable to continue their control on intra-extracellular exchanges (Soddu and Gioia, 1979; Glinka and Reinhold, 1972), leaving the structure of the matrix as solely responsible for the slowing down of the diffusion process.

It seems therefore quite unlikely that this approach could be used as the only predicting tool for living systems. It is obvious that the cells play a role in the overall behavior of the diffusion and that this effect should be taken into account in any model.

7.1.3 Application of the theory of irreversible thermodynamics: the interaction approach

The need to incorporate the influence of the living cells in the diffusion model led us to use an approach based on the principles of the theory of *irreversible thermodynamics* to describe the interaction of these entities with their surrounding medium. For instance, during the osmotic dehydration process, the diffusion occurring in the biological tissue affects strongly the chemical potential equilibria between the cells and their immediate extracellular solution. Fluxes are then generated in response to these changes in chemical potentials across the cell membranes. The cells can then be seen as *sinks* or *sources* that control the overall or macroscopic diffusion in the tissue matrix, since, depending on their membrane *permeability* characteristics, they will either pick up or lose water and solutes modifying continuously the composition of the extracellular solution. The cells are therefore no longer only obstacles but *living units* that have some control over their surroundings.

The theory of irreversible thermodynamics is particularly suitable for theoretical and modeling studies involving this kind of interaction since it permits construction of phenomenological relations between the fluxes and the forces that are present in such irreversible process. A discussion of the theory and of its use in the derivation of phenomenological relations describing transmembrane transport phenomena has been given in detail in Chapters 2 and 3 and will therefore not be repeated here. Let us just mention that the previous analysis, which was carried out by considering a single cell exposed to an osmoticum of constant composition, is directly applicable in the present case since, as far as each individual cell composing the tissue is concerned, the local extracellular solution surrounding it acts as such an osmoticum with the only difference that its composition is not constant. The reader can now foresee that our model should have provision for describing not only the intra-extracellular exchanges but also the transient behavior of the extracellular solution.

7.2 Mass transfer in plant storage tissue

As illustrated in Figure 7.1, there are two accepted pathways that water and/or solutes can follow while traversing plant storage tissues (Moorby, 1981; Nobel, 1983): the *apolasm pathway* or *apoplast*, which is *exterior* to the plasmalemma or cell membrane and can be described as diffusion of molecules in the cell walls and the free space between cells, and the *symplasm pathway* or *symplast*, which is *interior* to the plasmalemma and is characterized by a movement of molecules from one cell into another by means of small channels called *plasmodesmata* (singular: *plasmodesma*). Both routes are believed to be important and their respective contributions to the overall diffusion process are related to the nature of the tissue.

7.2.1 Apoplastic transport

It is well accepted that the cell wall space may serve as a phase or pathway for mass transport in plant tissue (Lüttge and Higinbotham, 1979). Movement in the cell wall is determined by the width of the interfibrillar and the intermicellar spaces and by the kind and concentration of charged ions fixed there (Donnan exchange). Transport in the cell wall phase can be separated kinetically from transport across the plasmalemma, i.e. from transport into the interior of the cell, with the former offering in general less resistance to diffusion than the latter (Briggs, 1957; Cram, 1968). This is attributable to the highly specialized

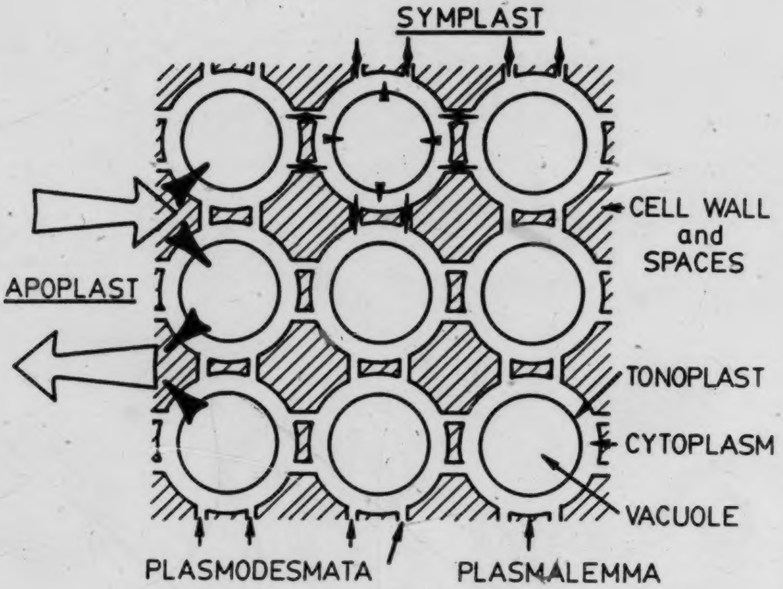
SYMPLAST
APOPLAST
CELL WALL and SPACES
TONOPLAST
CYTOPLASM
VACUOLE
PLASMODESMATA
PLASMALEMMA

Figure 7.1 Mass transport pathways in plant storage tissue.

nature of the cell membrane contradistinctively to the relative inertness of the apoplast complex. Indeed, experiments have demonstrated that apoplastic transport is a *passive* process, exclusively due to *physical* driving forces, as opposed to transmembrane transport which has been shown for some substances to be based on *metabolic* phenomena (Lüttge and Higinbotham, 1979; Hall and Baker, 1980).

7.2.2 Symplastic transport

As disclosed previously, symplastic transport is defined as any transport that occurs *within* the plasmalemma, in the continuum formed by plasma bridges or plasmodesmata linking cellular cytoplasms together (Moorby, 1981; Nobel, 1983). These bridges are fine, membrane-flanked, cytoplasmic threads that pass from a cytoplasm, through a pore in the cell wall, directly into the cytoplasm of a second cell. The plasmodesmata are not simple aqueous channels between cells. They have complex ultrastructural characteristics, the functions of which are not yet fully understood (see Robards, 1976). Nevertheless, the presence of those channels can provide a particularly effective pathway for water and solute(s) movement between adjacent cells where permeability barriers in the form of either cell walls or membranes can be avoided.

A modern description of symplastic transport based on the principles of the theory of irreversible thermodynamics was proposed by Tyree (1970; see also Anderson [1976]).

Briefly, the author uses the concepts of the theory to derive phenomenological equations that describe the transport of substances through the plasmodesmata based on *macroscopic permeability coefficients* characterizing the overall resistances encountered when molecules negotiate this pathway. This approach is analogous to assimilating the plasmodesma complex to an *equivalent membrane* having the permeability characteristics of the channel. Tyree has quantified, at least in order of magnitude, the values of these permeability coefficients, based on the parameters describing the plasmalemma permeability. He has shown that the "plasmodesma membrane" was far less restrictive than the plasmalemma, with the result that the symplast appears to constitute the pathway of least resistance for the diffusion of water and all small solutes.

In his derivation, Tyree used nonequilibrium states *within* the symplast as driving force for symplastic transport. The causes for the build-up of such nonequilibria can be of complex nature. Membrane transport processes, as well as synthesis and degradation reactions, are probably responsible for establishing most of the gradients encountered within the symplast. Symplastic transport has a tendency to equal out those gradients so that, as such, it is of a *passive* nature, that is, *molecular diffusion* is the predominant mechanism of transport across the plasmodesmata (see Robards, 1976).

7.3 Review of existing models

Although dehydration has been for millenia an efficient way of extending the storage life of foods and grains, it was not until the beginning of this century that experiments have been undertaken to explore the mechanisms controlling water removal from such porous structures (Chirife, 1983). Much of these efforts seem however to have been directed toward the study of moisture transport in the context of *air dehydration*, which represents by far the most common approach for reducing the water content of a medium where water is entrapped in a solid matrix. It is therefore not surprising to see an enormous amount of information available on the fundamental aspects of this process, with, in the past few years, some emphasis on the modeling of heat and mass transfer in cellular tissues undergoing drying (see Mujumdar, 1980, 1983).

Comparatively little work has been done, on the other hand, on the fundamentals of *osmotic dehydration* for similar structures. Existing models are mostly based on the assumption that the observed macroscopic mass transfer can be described by the simplified unsteady state Fickian diffusion model (equation 7.3). They are therefore of *correlative* nature (see section 7.1.1) and, as such, are limited to the studies of interest since they are based on apparent diffusion coefficients which are found, in the *least-squares* sense, to describe the observed diffusion phenomena (Hawkes and Flink, 1978; Conway, Castaigne, Picard and Vovan, 1983).

The inclusion of a formal description of the tissue structure as part of the diffusion model was examined by Soddu and Gioia (1979) in their study of sugar leaching from sugar beets immersed in water. Rotstein and Cornish (1978a,b) and more recently Crapiste, Whitaker and Rotstein (1984) introduced similar descriptions in their studies of air dehydration of cellular foodstuffs.

It is however in animal and plant physiology that most of the literature concerning modeling of osmotic transport phenomena at the *cellular* level can be found. Let us mention in particular the important contributions of numerous workers to the analysis of water and solute(s) transport in epithelial tissues (Wienstein and Stephenson, 1979; Liebovitch and Weinbaum, 1981) who developed models which not only describe the interaction of the epithelium cells with their environment but also give a detailed description of the local osmotic flow in the extracellular channels. The reader should notice here the close analogy which exists between the mechanistic elements considered in the above epithelium models and the characteristics which we propose to introduce in our microscopic description of mass transport in plant storage tissues (see section 7.1.3). We will return to this point later on.

The first major effort to develop a quantitative description of osmotic mass transport in plant tissues based on *cellular* properties is attributable to Philip

(1958a,b,c). His analysis was however restricted to describing exclusively the movement of water in a linear aggregation of cells since the osmoticum solutes were assumed to be impermeable to the plasmalemma. Molz and Hornberger (1973) extended Philip's model to include a permeable solute and used the practical equations for mass transport across biological membranes developed by Kedem and Katchalsky (1958) from the theory of irreversible thermodynamics (see section 2.2.4) and elaborated for plant cells by Dainty (1963), to describe the mass transfer in a similar structure. Yet, both models were relatively limited since they considered the mass fluxes as having to traverse the cell membranes in series, from surface to interior (or vice-versa), with no provision for possible contribution of apoplastic and/or symplastic transport in the overall diffusion phenomenon.

In a subsequent paper, Molz and Ikenberry (1974) improved the description by developing a model which allowed for apoplastic transport, i.e., for mass fluxes occurring in the cell walls without necessarily traversing the cell membranes. Molz (1976) eventually completed the model by accounting formally for symplastic transport along with apoplastic and transmembrane transfer. Restricting his study to water movement exclusively, the author used an *electrical circuit analog* to characterize the resistances in the various flow paths.

In all the aforementioned works it was assumed, in order to simplify the mathematical treatment of the models, that, since the individual cells could be considered much smaller than the aggregation, the anatomy of the tissue could be assimilated to a *homogeneous continuum*, allowing the local continuity equations to be integrated across the whole structure.

Molz and coworkers (Molz, Kerns, Peterson and Dane, 1979), on the other hand, employing the same circuit analog approach, proposed a model that was used to study the water relations between the intra- and extracellular phases of several plant cells in series. It is interesting to note that, contrary to the previous works, the model devised by these authors included explicitly some of the discrete cellular geometry and anatomy of the tissue being studied. The behavior of each individual cell could accordingly be simulated.

More recently, McCoy and Boersma (1984) applied the principles of *continuum mechanics* to formulate mathematical expressions for the transport of water and solute along the direction of deformation in plant tissue. These derivations are based upon the explicit consideration of the tissue as a mathematical continuum composed of a cell wall matrix, water and solute phases. It is worth pointing out that this model describes not only mass transfer but also *momentum* transfer phenomena and takes into account the coupling between both processes. The utilization of continuum mechanics has nevertheless the drawback that it leads to a macroscopic description of the tissue with no possibility of simulating the behavior of its cellular components.

7.4 Summary

From the above discussion it appears that the description of the mass transfer in plant storage tissues should consider both the apoplasm and symplasm pathways as probable routes for mass transport. Since it is quite unlikely that the osmotic behavior of the tissue could be approximated exclusively from the behavior of the constitutive cells (Philip, 1958c), that is, that the osmotic phenomena would be limited by the cells themselves and not partly by the resistance of the paths communicating with the external (osmotic) solution, we will have to conceive a model that takes into account:

1. The primary resistance to propagation in the apoplasm and symplasm pathways, which alters the homogeneity of the phenomenon and leads to the formation of internal gradients, and
2. The resistance to diffusion residing in the membrane enclosing the cells.

The global picture of mass transport in such a structure can then be seen as a concomitant diffusion of solutes from/to the bathing solution through the apoplast and symplast, coupled with local exchanges between intra-

and extracellular phases.

The approach adopted in this work is therefore more in line with the model presented by Molz *et al.* (1979), in the sense that we propose to provide a *microscopic* description of the mass transport phenomena in plant storage tissue. Our model will offer however some new features which are lacking in the representation proposed by Molz *et al.* (1979), that is, we will consider diffusion of permeable and impermeable solutes in the matrix, shrinkage of the whole structure along with other novel characteristics that will be disclosed in the following chapter.

8. MATHEMATICAL ANALYSIS OF MASS TRANSFER PHENOMENA IN PLANT STORAGE TISSUE: DEVELOPMENT OF THE MODEL

8.1 Mathematical representation of the tissue

To approach mathematical modeling of a diffusion process from a deterministic point of view, the physical dimensions and shape of the system must be defined. In the case of plant tissue, it is easy to imagine the difficulty of the task, especially if one attempts to model *exactly* the real structure. We have to bear in mind that the degree of sophistication needed is dependent on the precision that can be achieved in monitoring the tissue behavior and, since it is quite limited, a model which simulates those aspects of interest to the degree necessary for the study should be valid.

8.1.1 The average unit cell

It is well known that a relative coherence exists in plant storage tissues, particularly in the parenchyma of these roots (Esau, 1940; Poole, 1976). However, the random arrangement and the size range of the constitutive cells render a detailed description of the structure still difficult. That is why it is necessary to simplify the complexity of the system by generating an "*idealized*" copy of the structure based on typical properties of the real medium. By doing so, we ensure that the system being described is truly *isotropic* and *homogeneous*, which facilitates the development of the model.

The procedure for normalizing the properties of the structure usually consists in employing the *average* properties and characteristics of the tissue to generate a representative unit volume which is assumed to be essentially a *microrepresentation* of the whole system (see Neale and Nader, 1973; Crapiste *et al.*, 1984). In this work, we used the above approach to devise what we will refer hereafter to as the *average unit cell*. The procedure is as follows.

Figure 8.1a depicts the simplified structure of a typical plant storage tissue (superscript t). It consists of 2 main parts: firstly, the *extracellular volume* or *interstitium*, Vi^t, comprising the *cell wall volume*, Vw^t, and the *intercellular free space volume*, Vs^t; and, secondly, the *cellular volume*, Vc^t, which is formed of a "*solution*" or *osmotic volume*, Vo^t, and a *nonosmotic volume*, Vd^t (see section 4.1.1). It is important to note that our simplified picture considers the plant cell as a *simple osmotic system* (see section 2.2.3), that is, that the cytoplasm, the tonoplast and the vacuole form a *single unit* whereas the plasmalemma or cell membrane acts as the (semi)permeable barrier. The *intracellular volume* or *cellular volume* is therefore all the volume *interior* to the plasmalemma and is assumed to be a *homogeneous* thermodynamic phase, *isobaric* and of *uniform concentration* (perfect mixing; see Tyree, 1970). The permeability properties of the plasmalemma are

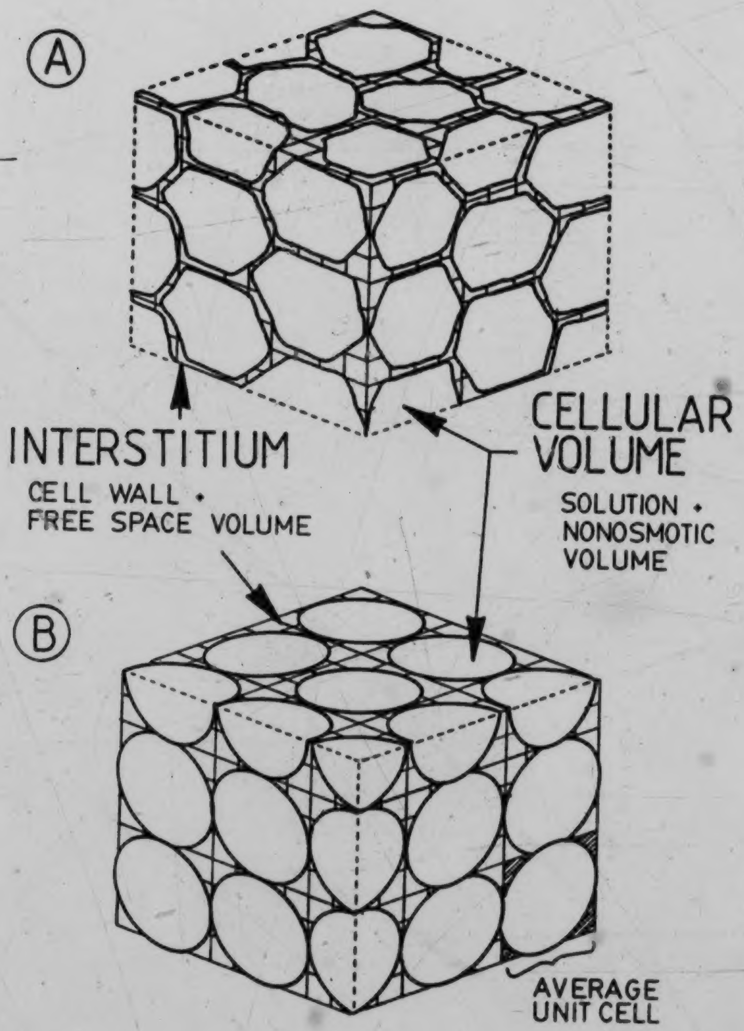
A
INTERSTITIUM
CELL WALL +
FREE SPACE VOLUME
CELLULAR
VOLUME
SOLUTION +
NONOSMOTIC
VOLUME
B
AVERAGE
UNIT CELL

Figure 8.1 Simplified structure of a typical plant storage tissue (a) and corresponding average analog (b).

assumed to represent the *total* of the resistances to be encountered in any transfer between intra- and extracellular phases.

As can be seen, the tissue can be assimilated to a relatively homogeneous swarm of cells possessing a *porosity* or *void fraction*, ϵ', that we will define as the fraction of the total tissue volume, V', which is not cellular volume, that is:

$$\epsilon' = Vi' / V' = \phi s' + \phi w' \qquad [8.1]$$

where $\phi s'$ and $\phi w'$ are, respectively, the volume fractions of the tissue intercellular free space and cell wall volume, given by:

$$\phi s' = Vs' / V' \text{ and } \phi w' = Vw' / V' \qquad [8.2]$$

If we now suppose that the parenchymatous cells are spherical[12] and choosing a *reference* sphere having the *average diameter* of the population of cells composing the studied tissue (which can be determined by microscopic observation), we can generate an *average unit cell* (superscript 1) consisting of the reference sphere representing a *unit cellular volume*, Vc', together with its associated *unit extracellular volume*, Vi'. The latter volume is calculated by employing the tissue void fraction, ϵ', that is:

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[12] The cells are in reality *polyhedral*. This assumption of sphericity is however not too limiting in view of other uncertainties inherent to the model which have far more impact.

$$Vi'_0 = (\epsilon'_0 \, Vc'_0)/(1 - \epsilon'_0) \qquad [8.3]$$

where the subscript 0 refers to a reference state of the tissue (usually full turgor), such that the average unit cell can be postulated to be a *microstructural analog* of the whole tissue. This last statement is crucial in the present development since it implies that, by virtue of the concept of the average unit cell, the analysis of mass transport in the entire structure is transposed from the usual global description down to the analysis of the behavior of representative microscopic units.

To make the mathematical treatment feasible we will consider an "idealized" model of the parenchymatous structure of plant storage tissue, as represented in Figure 8.1b (Nilsson, Hertz and Falk, 1958). We will suppose that the real tissue can be approximated by a *homogeneous* and *isotropic cubic* arrangement of average unit cells, i.e. that each unit is in contact with six neighbors. It is important to realize that the introduction of the average unit cell concept does not imply that the idealized analog of a given volume of "real" tissue contains an equivalent number of cells. The number of constitutive units in the analog, $\#c'$, is in fact determined by dividing the tissue volume, V'_0, by the volume of the average unit cell, V'_0, at the reference state, i.e.:

$$\#c' = V'_0 \,/\, V'_0 = V'_0 \,/\, (Vc'_0 + Vi'_0) \qquad [8.4]$$

The approach is therefore *per se* a *physical volume averaging* process by which the macroscopic characteristics of the tissue are transmitted equally to its constituents. That is what will be considered in the next section.

Properties and characteristics of the model system

We will present in the following analysis some properties and characteristics of the tissue that we wish the model to describe. The reader will notice that some aspects refer to the behavior of the system as a whole whereas others are dealt with at the level of the average unit cell. It must be remembered however that, since the average unit cell is supposed to be a microrepresentation of the entire structure, the concepts introduced here can be applied *interchangeably* to describe both entities. Let us first consider the description of the apoplastic transport.

In the model under consideration, we propose to use the *obstacle* approach (see section 7.1.2) to describe the resistances that could be encountered by the diffusing molecules in the *apoplastic volume*. Let us recall that the apoplast is the transport pathway comprising the cell wall and the intercellular free space continuum. It is therefore identical to the extracellular volume or interstitium. Three main parameters will be employed to characterize this pathway: the *tissue void fraction*, ϵ', which has already been introduced, the *interstitium geometrical tortuosity*, τi, and the *apparent interstitium diffusibility*, ψi.

The *tissue void fraction*, ϵ', is by definition (equation 8.1) a measure of the fraction of the total volume of the tissue that can be considered *apoplastic volume.* It is also equivalent, in this system, to the *average fraction free cross-section* (Sherwood, Pigford and Wilke, 1975), i.e., a measure of the fraction of the total exposed surface of any cross-section of the tissue normal to the macroscopic direction of flow which is available for mass transport. It permits therefore the evaluation of the *effective transfer area,* since it describes the reduction in diffusional space due to the presence of physical interferences, the cells in this case. This parameter is employed, usually in the context of the obstacle approach, to generate reduced apparent diffusivities from solution coefficients which are based on *unit total transfer area.* This correction factor can however be introduced in an *implicit* form to achieve the same goal if we devise the model such that the extracellular transport is considered no longer in relation to the total exposed surface but exclusively with respect to the *effective transport continuum,* i.e. the apoplast. This then becomes equivalent to expressing the solution diffusivities with respect to the *surface of apoplastic transfer.* We will examine this approach in more detail in sections 8.1.2 and 8.2.2.

The second parameter to be considered is the *interstitium geometrical tortuosity,* τi, which is defined, in the present context, as the ratio of the actual distance that a molecule has to travel in the tissue, as part of a *macroscopic unidirectional* flow, as compared to the distance covered normal to the surface. This factor does not consider the microstructural properties of the interstitium complex. It is only an approximation of the length of the path to be negotiated by a molecule diffusing in an *empty* space around a typical cell, i.e., to go from one side to the other in a *global unidirectional* flow.

For the cubic arrangement of spherical cells introduced previously, τi is then given by the ratio of the real distance covered around a cell, i.e., half its circumference, to the distance covered normal to the surface, z, that is:

$$\tau i = (\pi \; dc'/2) \; / \; z = \pi \; / \; 2 \qquad [8.5]$$

since dc', the diameter of the sphere representing the unit cellular volume, equals z. Let us note that τi represents the most probable increase in distance to be expected on average for molecules diffusing in a swarm of spherical cells having a cubic packing (τi is obviously dependent on the arrangement selected to describe the cellular structure). Let us point out furthermore that, if we assume that the cells are *elastically independent* and shrink or swell *isotropically*, the interstitium geometrical tortuosity remains constant. This factor, which again is used usually to generate apparent diffusivities (see section 7.1.2), will be introduced in the model, as for the void fraction, in an *implicit* form, to be detailed in section 8.1.2.

The only parameter which in fact will be employed *explicitly* to correct the solution diffusivities is the *apparent interstitium diffusibility*, ψi. It is introduced in order to take into account the effect of the *microstructural properties* of the apoplast, which can hinder the diffusion process along the pathway.

Let us consider the interstitium, the *cell wall* volume in particular. It can be seen as a network of cellulosic fibers which cross-linking creates voids and spaces in which diffusion can occur. It is therefore possible to characterize the resistance to diffusion in that environment by introducing a *cell wall void fraction*, ϵw ($0 \leq \epsilon w \leq 1$), similar to the one introduced earlier, defined as the ratio of space through which the molecules can diffuse in the cell wall complex to the total volume of the cell wall. Concomitantly, a *cell wall tortuosity*, τw ($\tau w \geq 1$), can be defined as the ratio of the actual distance covered in the complex as compared to the distance that would be travelled in an *empty* space. Both factors can then be used to correct the solution diffusivities in a manner analogous to the one introduced in section 7.1.2 (equations 7.5 and 7.6), that is, for diffusion of the i-th species in the cell wall:

$$Đ_i = \psi w \, D_i \; ; \; 0 \leq \psi w \leq 1 \qquad [8.6]$$

where, as before, $Đ_i$ and D_i are, respectively, the apparent pseudo-binary diffusivity and the solution diffusivity of component i, while ψw is the *apparent cell wall* *diffusibility*, assumed to be the following function of ϵw and τw:

$$\psi w = \epsilon w / \tau w \qquad [8.7]$$

The hindrance effect of the interstitium as a whole is a function of the combined resistances of both parts of the extracellular continuum and is more precisely defined (for any average unit cell, assuming a *parallel* effect) as:

$$\psi i' = [(\phi s' \ \psi s) + (\phi w' \ \psi w)]/(\phi s' + \phi w') \qquad [8.8]$$

where, as can be seen, we have extended the partition of the tissue interstitium to its average unit cell analog (superscript 1), i.e., $\phi s'$ and $\phi w'$ are, respectively, the average unit cell intercellular free space and cell wall volume fractions, and ψs is the apparent diffusibility of the intercellular free space volume.

Since there is negligible resistance to be expected in the latter volume, i.e., $\epsilon s \cong 1$ and $\tau s \cong 1$, leading to $\psi s \cong 1$, equation 8.8 can be rewritten as:

$$\psi i' = [\phi s' + (\phi w' \ \psi w)]/(\phi s' + \phi w') \qquad [8.9]$$

where by definition $\phi s'$, $\phi w'$, ψw and $\psi i'$ can take values from 0 to 1, inclusive.

As can be seen, the apparent interstitium diffusibility is a function not only of the intrinsic diffusibility of each part of the extracellular continuum but also of the fraction occupied by each component. It is therefore possible to simulate, as part of the model, the changes in resistance to diffusion that are likely to occur in the different stages of osmotic dehydration (to be discussed below) where, for example, there might be an increase in intercellular free space volume due to the retraction of the plasmalemma from the cell wall. Let us point out here that is it assumed that the cell wall structure is sufficiently rigid to ensure that its total volume remains constant, even though the whole tissue might shrink. This assumption is therefore equivalent to supposing that any volume gain or loss of the whole structure is attributable to changes in the intercellular free space and cellular volume, respectively.

Turning now to symplastic transport, i.e. transport which is interior to the plasmalemma of two adjoining cells, we will employ a measure of the total area available for transfer to characterize this pathway. More precisely, we will use the proportion, αp, of cell membrane (plasmalemma) occupied by plasmodesmal bridges, that is, the total surface of the presumptive conducting channels expressed as a proportion of the total membrane area, to weight the relative importance of this route as compared to trans-membrane transfer. Hence, the *total symplastic transfer area*, Ap' (for any average unit cell), will be given by:

$$Ap' = \alpha p \, Ac' \qquad [8.10]$$

where Ac' is the area of the sphere representing the unit cellular volume.

It remains finally, before concluding this section, to describe the general behavior of a typical unit cell undergoing osmotic dehydration and how it relates to the whole tissue behavior. Referring to Figure 8.2, three main stages will be considered (see Nobel, 1983):

1. From *full turgor* to *incipient plasmolysis*. At full turgor the cell is under internal pressure, i.e. due to intracellular hydrostatic pressure build-up, the cell membrane is stretched and forces the surrounding cell wall to touch the neighboring cells with minimum extracellular open spaces. As water and solutes are lost by the cell, we will suppose that the total cell membrane surface area decreases proportionally to the cellular volume loss following a 2/3rds power dependence, i.e. for *isotropic* changes:

$$(Ac' / Ac'_0) = (Vc' / Vc'_0)^{2/3} \qquad [8.11]$$

where the subscript 0 indicates the conditions at full turgor which, by virtue of the definition of the average unit cell, are the same for all the cells composing the tissue. Concomitantly, any change in *cellular* volume will be assumed to affect the *total* volume of the average unit cell, V', whereas the extracellular volume, Vi', stays constant, that is:

$$dV'/d\theta = dVc'/d\theta \text{ and } dVi'/d\theta = 0 \qquad [8.12]$$

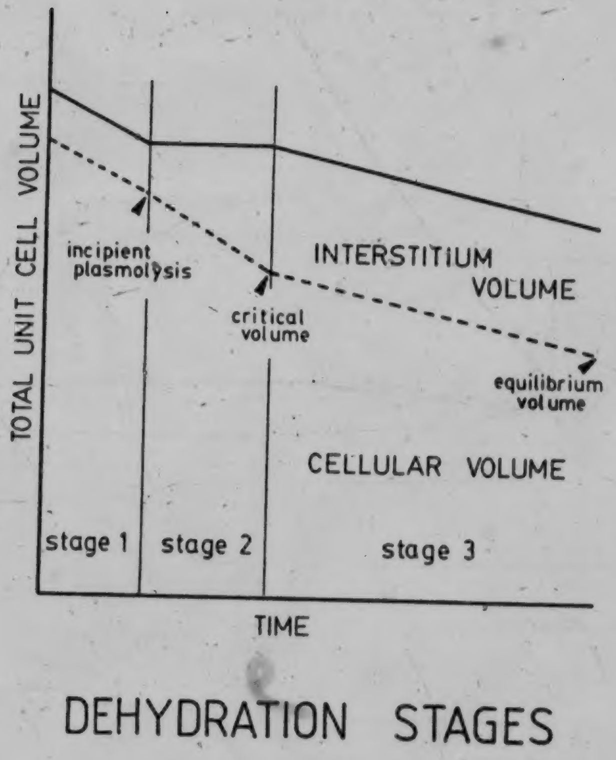
TOTAL UNIT CELL VOLUME
incipient plasmolysis
critical volume
INTERSTITIUM VOLUME
equilibrium volume
CELLULAR VOLUME
stage 1
stage 2
stage 3
TIME
DEHYDRATION STAGES

Figure 8.2 General behavior of a typical unit cell undergoing osmotic dehydration.

where θ is the time variable.

2. From *incipient plasmolysis* to the *critical cell volume*. As soon as incipient plasmolysis (loss of turgor) is reached, any further loss in cellular volume will be assumed to be compensated for by a proportional increase in *intercellular free space volume* as the cell membrane pulls away from its surrounding cell wall. Since, by definition, the interstitium volume, Vi', is the sum of the cell wall volume Vw' (assumed to stay constant) and the intercellular free space volume, Vs', the above rearrangement can be expressed as:

$$dVi'/d\theta = dVs'/d\theta = -dVc'/d\theta \qquad [8.13]$$

where, again, we have extended the partition of the tissue interstitium to its average unit cell analog. During that period, the total volume of the unit stays constant, i.e. $dV'/d\theta = 0$, as does the cell membrane surface area (the latter assumption supposes that the plasmalemma folds on itself [see section 4.1.1]).

3. From the *critical cell volume* to the *end* of the dehydration process. In order to account for the *shrinkage* phenomenon which is observed in most dehydration processes, it is assumed that the cell can reach a point, the *critical cell volume*, where the extent of volume loss is such that the cell membrane starts to pull the cell wall inward from its points of anchorage to that structure. The whole complex therefore collapses. For that period we will assume that the

extracellular volume stays constant such that the *total* volume changes proportionally to the loss in cellular volume (equation 8.12).

The dynamic of these phenomena can be generalized if one introduces a *compliance factor*, κ, which describes how *cellular* volume changes affect the *extracellular* volume of the unit cell. The above relations can then be represented as follows (for all stages) for the change in interstitium volume:

$$dVi'/d\theta = \kappa\, dVc'/d\theta \qquad [8.14]$$

and accordingly for the total unit volume, since $V' = Vi' + Vc'$:

$$dV'/d\theta = (1 + \kappa)\, dVc'/d\theta \qquad [8.15]$$

where, for stages 1, 2 and 3, the compliance factor takes the values of $\kappa = 0$, -1 and 0, respectively.

The concept of the average unit cell assumes its importance when one wishes to extend the above analysis to the entire structure. Indeed, by monitoring the behavior of each individual cell, it then becomes possible to follow the extent of structural reorganization within the tissue[13], and, therefore, to draw a complete picture of the whole system by "*integrating*" the observed individual changes over the total number of constitutive units. For example, the volume change of the tissue will be given by:

---

[13] We assume that all the rearrangements that the tissue experiences during the dehydration process are *reversible*, i.e. that the medium is capable of returning to its initial condition if we reverse the phenomenon.

$$dV^t/d\theta = \Sigma_j dV^{tj}/d\theta \; ; \; j = 1,2,\ldots,\#c^t \qquad [8.16]$$

where $\#c^t$ is the total number of average unit cells calculated using equation 8.4.

Relations 8.14 and 8.15 represent a very crude description of the deformation of the tissue during osmotic dehydration. One can obviously improve this treatment by including mechanical stress analysis and other features relating osmotic changes to momentum transport (see McCoy and Boersma, 1984). However, as shown by Suzuki and coworkers (Suzuki, Kubota, Hasegawa and Hosaka, 1976) and Lozano and coworkers (Lozano, Rotstein and Urbicain, 1983), the above approximations appear reasonable in the context of osmotic dehydration, in view of the fact that the process stays well within the range of moisture content at which the shrinkage of the tissue can be related directly to the net volume lost by the structure. The extent of dehydration to be considered in this work corresponds to lowering the moisture content of the tissue from an initial value of approximately 4 kg water per kg dry matter (assuming an initial moisture content for red beet or potato tissue of 0.8 kg water per kg fresh weight) to 3.2 kg water per kg dry matter in the case of red beet tissue or to 1.7 kg water per kg dry matter in the case of potato tissue (see section 9.1.1). This range of moisture contents is usually referred to as *high moisture region* (Rotstein and Cornish, 1978c).

8.1.2 The equivalent cylindrical unit cell

Although the properties and characteristics of the model system have been presented, the prediction of the transport processes within our idealized swarm of spherical parenchymatous cells requires further analysis to lead to a usable predictive model. More precisely, due to the mathematical complexity of describing diffusive flows within the proposed simplified structure, it appears necessary to introduce an even simpler representation of the system. This further simplification comes also from the necessity to describe efficiently the behavior of a parenchymatous cell undergoing osmotic changes. Let us recall that these changes occur when a cell interacts with its surrounding extracellular solution in order to maintain its intracellular integrity. These osmotic transients in our case are caused by the diffusion in the root tissue of components of the external osmoticum which, due to their nature, disrupt the internal equilibrium of the cell as they move in.

The problem at this level is therefore to conceive a *realistic* model of a cell and its environment. The model must provide a means not only to permit a concentration gradient to exist in the immediate cell extracellular solution, i.e., that a cell could *"see"* on one side the diffusion front and start reacting to it while the other side would still be facing the original tissue solution, but also must allow, since *perfect mixing* of the intracellular phase is assumed, material to cross the cell membrane on the *entirety* of its surface of exchange in response to the generated differences in chemical potentials.

The *equivalent cylindrical unit cell* has been contrived in order to respond, among other things, to these constraints. The main advantage of this design is that it conciliates the restriction imposed by the *continuity* of the extracellular volume and the *discontinuity* created by the spatial arrangement of the cells in the tissue matrix. The development of the *equivalent cylindrical unit cell*, which will be discussed in the following sections, and, by extension, of a *hypothetical* parenchymatous tissue, was inspired by the work of Grabowski (1974) on osmotic weight transients in myocardium, which was based on the *Krogh capillary cylinder concept* (Krogh, 1930), and by the analysis of electrolyte transport across a simple epithelium by Weinstein and Stephenson (1979).

The Krogh capillary cylinder concept

August Krogh developed his concept after observing the anatomy of capillaries in striated muscle (see Figure 8.3). Krogh found that in a given portion of muscle the capillaries were dispersed with conspicuous regularity. Consequently, since the muscle could be considered *homogeneous*, a *single* capillary and the tissue surrounding it could be extracted and treated as a *representative sample* of the whole muscle.

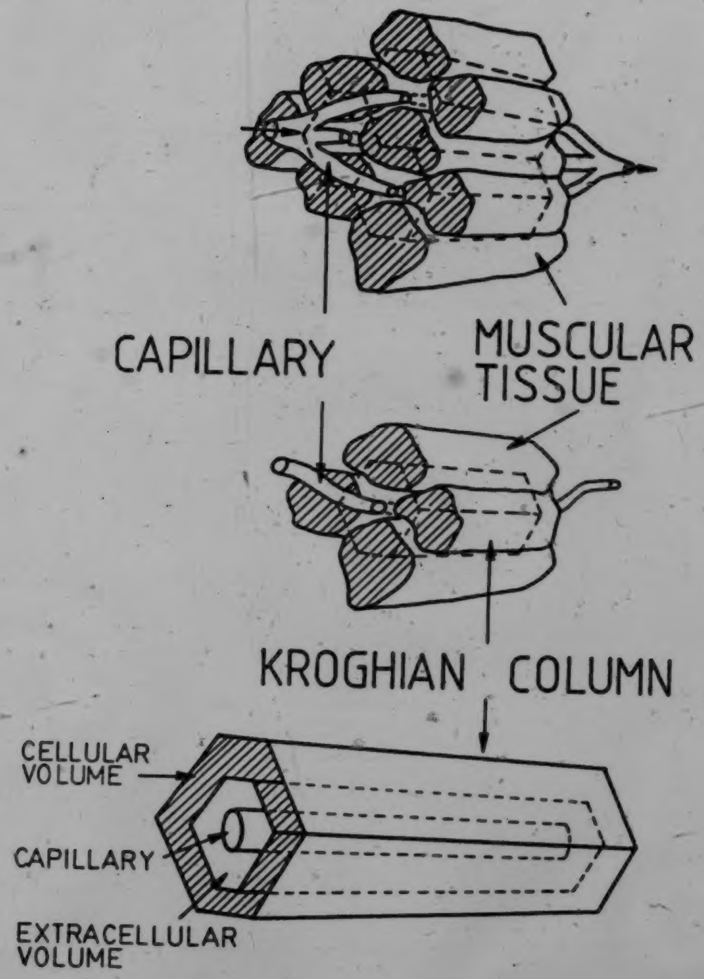
CAPILLARY
MUSCULAR TISSUE
KROGHIAN COLUMN
CELLULAR VOLUME
CAPILLARY
EXTRACELLULAR VOLUME

Figure 8.3 Krogh capillary cylinder concept.

Although strictly applicable to animal tissue, since it describes the reality of its structure, the concept of the Kroghian column was considered as a valuable alternative in approximating the cellular matrix of plant storage tissue. The idea is to take advantage of the homogeneity and isotropy of the idealized parenchymatous tissue developed earlier (see Figure 8.1b) to assimilate it to a Kroghian-like system. To do so, the extracellular continuum of a *characteristic portion* of the plant tissue is considered analogous to the single capillary of the Kroghian column, whereas the cells which comprise this sample are compared to the tissue surrounding it. It then follows that, if we assume that the selected portion is truly representative of the whole, the behavior of the column is an exact representation of the behavior of the entire tissue.

The selection of this characteristic subsystem and consequently the usefulness of the proposed approach obviously depend on the type of mass transport phenomenon to be described by the model. Thus, if we confine the analysis to the description of *unidirectional bulk diffusion* in plant storage tissue, the most logical choice, especially in view of its strong analogy with the Kroghian column, is to select as a representative section (referring to Figure 8.1b) the chain formed by any *linear arrangement* of adjoining average unit cells *parallel* to the macroscopic direction of transfer. The length of this chain would then be determined by the geometry of the tissue under study, which in our case will be restricted to systems that can be assimilated either to a *semi-infinite* medium or to an *infinite slab* of known thickness. The whole structure is therefore represented by a *bundle* of cellular "strings", the behavior of which is assumed identical.

The geometrical transposition

Although the description of unidirectional mass transport in plant storage tissue is greatly simplified by the introduction of the Kroghian concept (since we can now deal with one cellular string to model the tissue behavior), the transfer within that column remains, at the cellular level, *multidimensional* considering the fact that the molecules have to travel in a linear arrangement of spheres. The phenomenon is also complicated by the *interactive* role played by the cells in controlling the intra-extracellular exchanges. The complexity of the problem can however be substantially reduced if we *linearize* the system by *transposing geometrically* each constitutive average unit cell to its *cylindrical equivalent*, which we will refer to as *equivalent cylindrical unit cell* (abbreviated ECUC hereafter). The process consists basically in generating *coaxial cylinders* which represent each part of the average units, such that by assembling them *linearly* we create two *continua* which can be seen as identical to the capillary of the Kroghian column and the tissue surrounding it (see Figure 8.3).

Let us consider, to illustrate the above process, one of the average unit cells composing the selected string and its inherent characteristics as described in section 8.1.1. The reader is referred to Figure 8.4 for a complete picture of the equivalent cylindrical unit cell and its components.

The first mensuration to be established is the *length* of the coaxial cylinders which comprise the ECUC, namely: the *cellular cylinder* representing the cellular volume, the *interstitium cylinder* representing the extracellular volume and the *buffer cylinder* which will be introduced below.

According to our decision to restrict the present study to the analysis of mass transport in a *semi-infinite* body or in an *infinite slab*, and since we are interested as part of the model to describe possible shrinkage of the structure (see section 8.1.1), we will relate the length of the ECUC to the *total volume* of the average unit cell, such that changes at that level for any unit comprising the column will be represented by an equivalent decrease (increase) in the length of its corresponding cylindrical cell. It is therefore assumed that, for a semi-infinite medium or an infinite slab, shrinkage is a *macroscopic unidimensional* process. As discussed in section 8.1.1, since shrinkage of the tissue can be related directly to the *net (total) volume* lost by the structure, it follows that for the two previous geometries, if we assume *isotropic* shrinking (swelling) of the average unit cells composing the representative column, the phenomenon can be legitimately represented by the

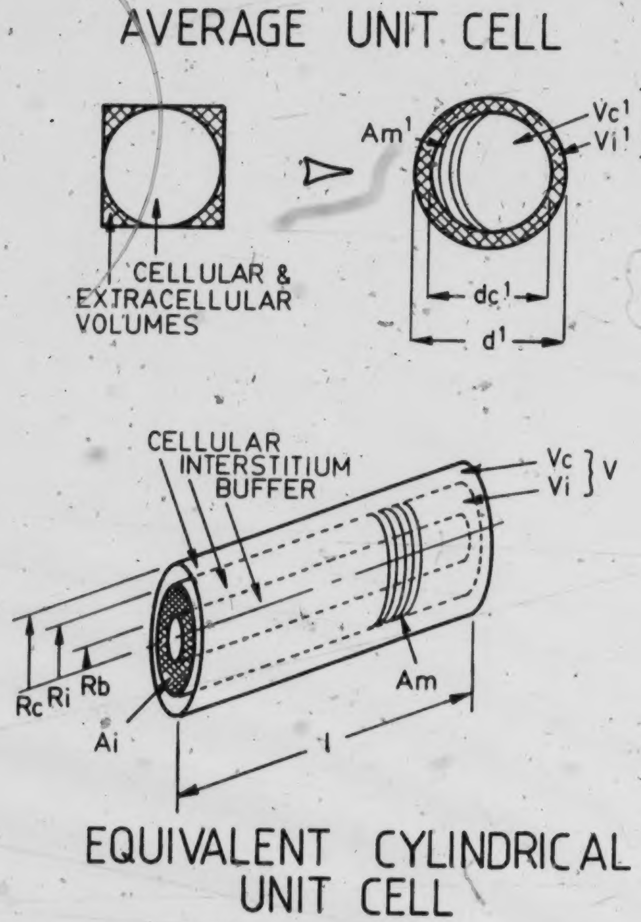
AVERAGE UNIT CELL
CELLULAR &
EXTRACELLULAR
VOLUMES
Am¹
Vc¹
Vi¹
V¹
dc¹
d¹
CELLULAR
INTERSTITIUM
BUFFER
Vc
Vi
V
Rc
Ri
Rb
Ai
Am
l
EQUIVALENT CYLINDRICAL
UNIT CELL

Figure 8.4 The average unit cell and the equivalent cylindrical unit cell.

movement of the system along *one* of its dimensions.

Hence, let ℓ, the length of the ECUC, be defined as the *diameter* of a sphere representing the *total* volume of the average unit cell, d', times the *square root* of the *interstitium geometrical tortuosity*, τi, that is:

$$\ell = d' \sqrt{\tau i} \qquad [8.17]$$

where by definition:

$$d' = (6\ V'/\pi)^{1/3} \text{ and } V' = Vc' + Vi' \qquad [8.18]$$

It can now be seen more precisely what was meant in section 8.1.1 by "introducing τi in an *implicit* form as part of the model". The inclusion of τi in equation 8.17, and later of the porosity, ϵ', as part of a similar relation, is governed by our desire to link these *geometrical* correction factors to the mensural properties of the ECUC and, accordingly, to leave $\psi i'$, the apparent interstitium diffusibility, the role of describing formally the hindrance effect of the interstitium complex.

The square root of the tortuosity is a necessary outcome of the requirements of *dimensional analysis* and the ensuing rules of *geometrical similarities* (Loncin and Merson, 1979), which state that, if we have a model and a *geometrically similar* prototype, the dimensionless concentration at *corresponding places* will be *identical* only when the *Fourier* numbers for mass transfer for both systems are equal. This means that, if we consider, for example, the value of the reduced concentration, say at a distance d' from the tissue surface, that is, on the other side of the first average unit cell, this concentration will be the same on the other side of the corresponding ECUC only if the Fourier numbers describing the mass transport in both cases are equal.

Therefore, assuming that the only factor affecting diffusion is the geometrical tortuosity, τi, for the first spherical average unit cell and the i-th diffusing species:

$$Fo(m)_i = \mathcal{D}_i \theta / (d')^2 \qquad [8.19]$$

where $Fo(m)_i$ is the Fourier number for mass transfer for species i, θ is the time variable and $\mathcal{D}_i$ is a corrected apparent diffusivity given by:

$$\mathcal{D}_i = D_i / \tau i \qquad [8.20]$$

where D_i is the solution diffusivity of component i.

Since we want τi to be implicitly part of the geometry of the ECUC, more precisely in the definition of its length, the Fourier number for the cylindrical cell will be given by:

$$Fo(m)_i = D_i \theta / \ell^2 \qquad [8.21]$$

where ℓ is the length of the first cylindrical cell.

Hence, since equations 8.19 and 8.21 must be equal to ensure geometrical similarity, inserting equation 8.20 in equation 8.19 and equating relations 8.19 and 8.21 leads to:

$$\ell = d' \sqrt{\tau i} \qquad [8.22]$$

which is identical to equation 8.17.

The second step in the development of the ECUC is the definition, in accordance with the following specifications, of the *radii* of the coaxial cylinders representing the components of the average unit cell:

1. Radius of the *interstitium cylinder*. Since the average unit cell is spherical, the surface of exchange between extracellular and cellular volume, that is, the area of the plasmalemma, Am' (excluding the proportion of cell membrane occupied by the plasmodesmata, αp [see section 8.1.1]), is given by:

$$Am' = (1 - \alpha p)\, \pi (dc')^2 \qquad [8.23]$$

where, as before, dc' is the diameter of the sphere representing the unit cellular volume. The radius of the interstitium cylinder, Ri, must therefore be, at any time[14], such that:

$$Am' \equiv Am = 2\pi \, Ri \, \ell \qquad [8.24]$$

i.e., Ri is calculated in order to make the area of the interface cellular-interstitium cylinders, Am, identical to the area of the plasmalemma[15] (see Figure 8.4), leading to:

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[14] Since all the mensurations are dependent on geometrical properties which are altered as osmotic changes progress, they are *point* functions in time (see section 8.2.4).

[15] For convenience we will adopt the notation introduced to characterize the properties of the average unit cell to describe identical properties of the ECUC. We will however

$$Ri = Am' / (2\pi \ell) \quad [8.25]$$

2. Radius of the *cellular cylinder*. Since the cellular cylinder represents the *cellular* volume of the average unit cell, Vc', its radius, Rc, must be such that, at any time:

$$Vc' \equiv Vc = \pi\ell\ (Rc^2 - Ri^2) \quad [8.26]$$

which yields:

$$Rc = \{[Vc'/(\pi\ell)] + Ri^2\}^{1/2} \quad [8.27]$$

3. Radius of the *buffer cylinder*. The buffer cylinder is introduced as a *geometrical artifice* to ensure that, since ℓ, Ri and Rc are now fixed, that the volume of the interstitium cylinder, Vi, is identical to its average unit cell equivalent, Vi', that is:

$$Vi' \equiv Vi = \pi\ell\ (Ri^2 - Rb^2) \quad [8.28]$$

leading to:

$$Rb = \{Ri^2 - [Vi'/(\pi\ell)]\}^{1/2} \quad [8.29]$$

As can be seen, the above design not only transposes the spherical average unit cell into another geometry but also *inverts* the whole system. The main reason for selecting this approach, besides the simplicity of the transformation, is the subjacent wish to restrict the simulation to the

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[18] (cont'd) drop the superscript 1, which is reserved to identify properties of the former unit (see List of Symbols).

analysis of *unidirectional bulk* diffusion in the tissue. This new arrangement and cell shape has the main advantage of *linearizing* the extracellular diffusion path at the level of each cell, which makes the ECUC similar to a Kroghian-like system. It is therefore possible to simulate the establishment of gradients of concentration "around" any given cell since all apoplastic transport processes are now described by diffusion in the linearized environment of the interstitium cylinder, with one end representing the "front" of the average unit cell and the other the diametrically opposite side. Hence, since the exchanges with the intra-cellular material (which are controlled by the differences in chemical potentials between a perfectly mixed cellular phase and an inhomogeneous extracellular solution), occur across the interface separating interstitium and cellular cylinders all along its length, one could observe on one end *efflux* of material while on the other, *influx* could take place, which is in fact the process considered by Philip (1958a,b,c) in his pioneering work, or by Molz and Hornberger (1973).

The inversion is also a way to respect the constraint of constant cell membrane surface area for a cell undergoing volume changes. Indeed, for fixed value of the length of the equivalent cell, one can observe (equation 8.24) that the surface area of the plasmalemma becomes directly related to the radius of the interstitium cylinder, Ri, which therefore prohibits any alteration to that value if the transfer area must be kept constant. However, as can be seen in Figure 8.4, the coaxial arrangement of the ECUC, with the cellular volume being represented by the outer cylinder and the buffer cylinder by the inner one, allows complete freedom in modifying the cellular and/or extracellular volume without altering Ri, since these changes can be implemented by varying the radius of the cellular cylinder, Rc, and/or the radius of the buffer cylinder, Rb (see equations 8.26 and 8.28).

From the preceding discussion, it should now be clear that changes in volumes and/or transfer areas of components of the average unit cell are reflected by corresponding changes in the radii and the length of the ECUC. The following approach will therefore be adopted to picture the different dehydration stages described in section 8.1.1:

1. When *total volume* changes, such as in stages 1 and 3, the length, ℓ, of the ECUC will be altered to simulate shrinkage, with the simultaneous reevaluation of the radii of the ECUC cylinders to maintain the characteristics of the unit (volumes and surface areas) which are not affected by the process.
2. Any *internal rearrangements* that do not affect total volume, such as change of interstitium volume versus change of cellular volume (stage 2), will be simulated by keeping ℓ *constant* and altering the radii of the ECUC cylinders. This approach is therefore geometrically in agreement with the assumption of relating the *unidimensional* movement of the

tissue undergoing shrinkage strictly to total volume change.

Another interesting feature of the proposed ECUC is that the ratio of the *interstitium* cross-sectional surface of exchange, Ai, to the *total* surface of the cylindrical cell normal to the macroscopic direction of diffusion, A, is equal to the *void fraction* of the average unit cell, ϵ', and, by extension, to the *average fraction free cross-section* of the system.

Referring to equation 8.3, the extracellular volume of the average unit cell is given at any time by:

$$Vi' = (\epsilon' Vc')/(1 - \epsilon') \qquad [8.30]$$

where we have extended the definition of the *tissue void fraction*, ϵ', to its cellular analog, that is (see equation 8.1):

$$\epsilon' = Vi' / V' \qquad [8.31]$$

Note that initially, i.e. at the reference state of the tissue, ϵ' and ϵ' are equal.

If we now insert equation 8.28 in equation 8.31 and use equations 8.26 and 8.28 to define V, the ECUC equivalent of the *total* volume of the average unit cell, V':

$$\begin{aligned} V' \equiv V &= Vc + Vi \\ &= \pi \ell \, (Rc^2 - Rb^2) \end{aligned} \qquad [8.32]$$

then the cell void fraction is:

$$\epsilon' = [\pi \ell \, (Ri^2 - Rb^2)]/[\pi \ell \, (Rc^2 - Rb^2)] \qquad [8.33]$$

which is equivalent to:

$$\epsilon' = Ai \, / \, A \qquad [8.34]$$

where Ai, the interstitium surface of exchange normal to the direction of diffusion, is given by:

$$Ai = \pi \, (Ri^2 - Rb^2) \qquad [8.35]$$

and A, the total surface of the ECUC normal to the direction of transfer, is defined as:

$$A = \pi \, (Rc^2 - Rb^2) \qquad [8.36]$$

Note that in all the above treatments the *buffer cylinder* was not considered since it is an artificial entity with no physical relation to the real tissue.

Equation 8.34 is particularly important for the derivation of the *continuity equations* depicting the mass transport in the proposed system (to be introduced in section 8.2). It reveals that the ECUC has the built-in property of allowing the extracellular diffusion phenomena to be described exclusively with respect to Ai, i.e. the *surface of apoplastic transfer*. This means practically that, to the extent that apoplastic transport is concerned, the cells *do not* exist as *physical interferences*, which renders the *explicit* use of ϵ', in a relation similar to equation 7.6, irrelevant as far as describing the influence of these obstacles on the transport phenomena. The reader can now observe that, since τi and ϵ' are *intrinsically* part of the *geometrical* properties of the model (equations 8.17 and 8.34), $\psi i'$ becomes the only entailed parameter to describe the characteristics of the apoplast complex.

The hypothetical parenchymatous tissue

From the redefined cellular unit developed from physical and mensural considerations, one can now generate a *hypothetical equivalent parenchymatous tissue* by replacing each average unit cell composing the medium by a cylindrical equivalent. The original real structure is now approximated by a fascicular arrangement of columns formed by the linear assemblage of equivalent cylindrical unit cells.

This design appears particularly appropriate for modeling unidirectional mass transfer in storage tissue since, as can be seen in Figure 8.5, the interconnected cylindrical cells provide an extracellular *continuum* approximating the apoplasm pathway of the real tissue, while still allowing the cells to remain independent units which are linked by the symplast.

8.2 The continuity equations

Let us now consider the equations describing the *isothermal* mass transport phenomena occurring in the proposed hypothetical tissue, more precisely, within the column selected as representing the entire system. Two basic processes will be described (see section 7.4), namely: the *cell interactions*, which will be modeled by relations based

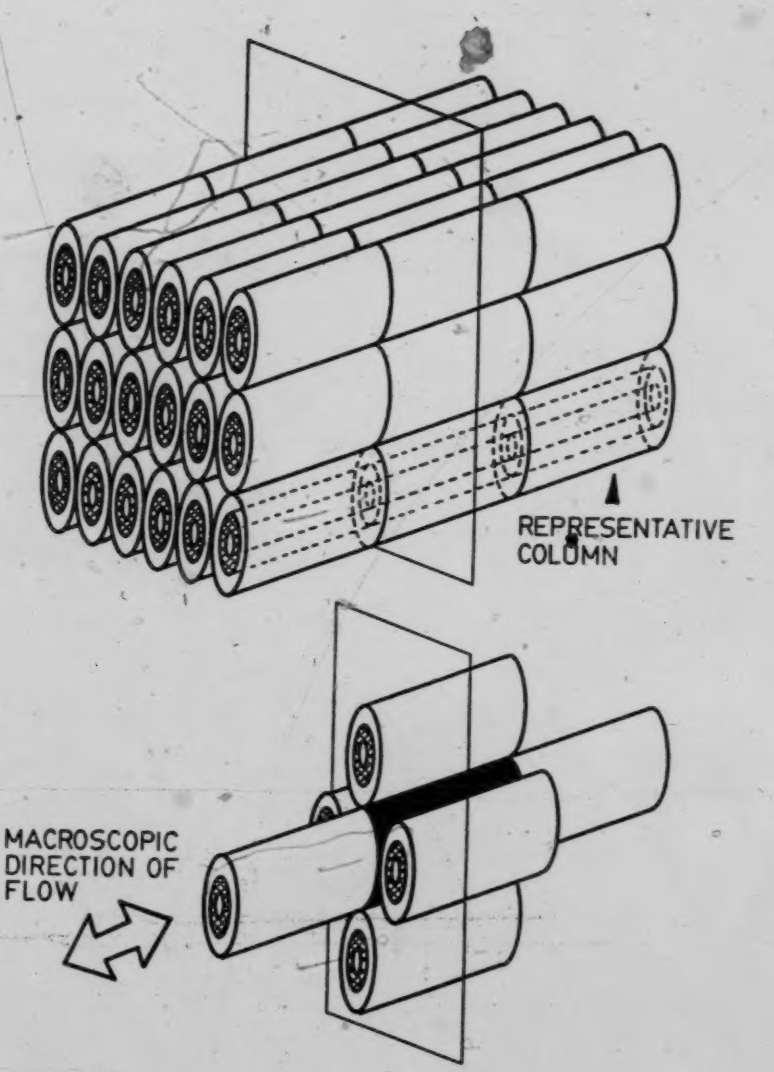
REPRESENTATIVE COLUMN
MACROSCOPIC DIRECTION OF FLOW

Figure 8.5 The hypothetical equivalent parenchymatous tissue.

on the theory of irreversible thermodynamics, and the *overall* or *bulk unidirectional* diffusion, which will be approached by relations associated with the extended form of the second order Fick equation.

8.2.1 Transmembrane exchanges

As discussed in section 7.1.3, the description of mass transfer across the plasmalemma complex and the plasmodesma pseudo-membrane will be based on the theory of irreversible thermodynamics, which, let us recall, allows one to write *phenomenological* equations describing such a process. Since the application of the above theory to transmembrane exchanges has been introduced in detail previously (see section 2.2.3 and Chapter 3), we will restrict the following discussion to presenting how the relations derived in the aforementioned sections have been adapted for the present analysis.

The discussion covers three main topics: firstly, the definition of the thermodynamic forces involved in transmembrane and symplastic transport; secondly, the description of the macroscopic phenomenological coefficients characterizing the membrane permeabilities and, thirdly, the definition of the flux equations. Note that the term *transmembrane transport* will indicate hereafter the transfer occurring across the *plasmalemma* complex whereas *symplastic transport* will refer to transfer across the plasmodesma pseudo-membrane.

Definition of the thermodynamic forces

Restricting our treatment to the transport of *nonelectrolytes*, the thermodynamic forces are defined in accordance with equation 2.25, which for a *plant cell* environment is given by:

$$\Delta\mu_i = \mathcal{R}T\Delta\ln a_i + \tilde{v}_i\Delta P \qquad [8.37]$$

where, as before, $\Delta\mu_i$ is the difference in chemical potential across the membrane for the i-th species, $\mathcal{R}T\Delta\ln a_i$ is the term related to the gradient of molecular potential and $\tilde{v}_i\Delta P$ describes the contribution to the force of the *hydrostatic pressure* difference between the phases separated by the barrier. Note that the last term is particular to plant cells, which can develop important gradients of hydrostatic pressure due to the presence of rigid cell walls surrounding them (see Nobel, 1983). Turning now to the system represented by Figure 8.6, let us consider the j-th member of the column of ECUC selected as a representative portion of the hypothetical parenchymatous tissue. To preserve the clarity of the following derivations, we will reserve the use of the superscript j (to indicate properties pertaining to the j-th cell) to cases where this symbol is essential for comprehension. The reader should therefore keep in mind that all the relations to be presented in the next sections describe the behavior of the j-th member of the representative column.

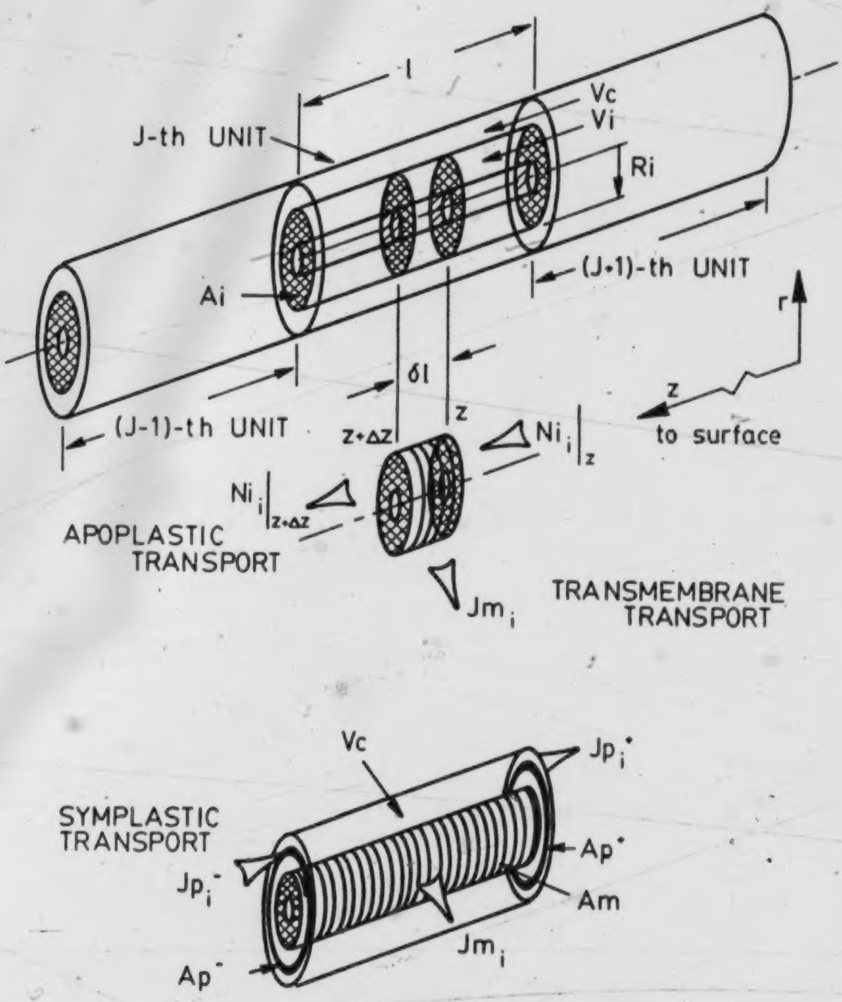
l
Vc
Vi
J-th UNIT
Ri
Ai
(J+1)-th UNIT
r
z
to surface
δl
(J-1)-th UNIT
z+Δz
z
Ni$_i|_z$
Ni$_i|_{z+\Delta z}$
APOPLASTIC TRANSPORT
Jm$_i$
TRANSMEMBRANE TRANSPORT
Vc
Jp$_i^+$
SYMPLASTIC TRANSPORT
Jp$_i^-$
Ap$^+$
Am
Jm$_i$
Ap$^-$

Figure 8.6 The j-th equivalent cylindrical unit cell and its environment.

For mass transfer across the *plasmalemma* of that unit, equation 8.37 will be approximated as follows:

1. Difference in *molecular potential*. As discussed in section 4.1.1, due to the difficulty of accessing the value of the *activity*, a_i, in complex multicomponent systems, we will adopt the approach proposed in the above section to approximate the first term of equation 8.37, namely, for the solvent water (index i = 1):

$$\mathcal{R}T\Delta\ln \mathit{am}_1 \cong -\mathcal{R}T\ \tilde{v}_1\ \Sigma_i(\mathit{cc}_i - \mathit{ci}_i) \qquad i = 2,3,\ldots,m \qquad [8.38]$$

where the summation runs for the m species present in the system once the tissue is immersed in the osmotic bath, i.e. the species initially present in the cellular and extracellular solutions and the species introduced by the osmoticum. Note that, contrary to the convention adopted in section 2.2.3, the difference operator, Δ, is defined as the difference between *intracellular* and *extracellular* quantities, i.e., in this case, the intracellular concentration of species i, cc_i, minus the interstitium concentration of that species, ci_i.

For all the species other than water, the difference in molecular potential across the plasmalemma will be given by:

$$\mathcal{R}T\Delta\ln \mathit{am}_i \cong \mathcal{R}T\ (\mathit{cc}_i - \mathit{ci}_i)\ /\ \bar{\mathit{cm}}_i \qquad i = 2,3,\ldots,m \qquad [8.39]$$

where $\bar{\mathit{cm}}_i$ is the *arithmetic* average of the concentration of the i-th species across the plasmalemma, i.e.:

$$\bar{c}m_i = .5\ (cc_i + ci_i) \qquad [8.40]$$

Let us recall that the above approximations are based on the assumption of ideality and diluteness of the system solutions. The question of ideality, as we have seen in section 4.1.1, can be handled reasonably well by the use of *apparent concentrations*. As for the diluteness, we propose to use the concept of *apparent cellular nonosmotic volume* to correct the equations. To that respect, it is interesting to note that, for plant cells, as discussed by Rotstein and Cornish (1978b), the latter assumption is not a heavy restriction since, even at a moisture content of 1 kg water per kg dry matter, the water mole fraction in the vacuolar volume (which can represent up to 90% of the total cellular volume) is still high (>0.9). Let us point out also the *local* nature of equations 8.38 and 8.39. As can be observed, although intracellular concentration, cc_i, has a *single* value (since perfect mixing of the intracellular solution is assumed), the interstitium concentration may vary continuously along the transfer area, which therefore renders the difference in molecular potential *dependent on position* over the entire length of the interface. We will return to this point in section 8.2.2.

2. Difference in *hydrostatic pressure*. The change of intracellular hydrostatic pressure is usually related to the change of cellular volume through a measure of the

reversible elastic properties of the cell walls known as *Young's modulus* or *elastic modulus*, ξ (Nobel, 1983; Dainty, 1976), that is:

$$dPc = \xi\, dVc/Vc \qquad [8.41]$$

where Pc is the hydrostatic pressure in the cellular volume in *excess* of the atmospheric pressure.

As discussed by Dainty (1976), the elastic modulus is in general *strongly* dependent on the internal pressure. Hence, if we assume a relationship of the form (Nilsson *et al.*, 1958):

$$\xi = \beta_1\, Pc + \beta_2 \qquad [8.42]$$

where β_1 is defined as the variation of ξ with respect to the intracellular hydrostatic pressure, Pc, that is $d\xi/dPc$ and β_2 is the value of the elastic modulus at *incipient plasmolysis*, i.e. when $Pc = 0$; and we integrate equation 8.41 with respect to the conditions at *full turgor* (*reference state*, subscript 0), the internal hydrostatic pressure becomes the following function of the cellular volume:

$$Pc = [Pc_0+(\beta_2/\beta_1)](Vc/Vc_0)\uparrow\beta_1 - (\beta_2/\beta_1) \qquad [8.43]$$

where the up arrow, ↑, indicates that the term on the left is to the *power* β_1. Note that, *at the reference state*, the cells composing the model column are *identical*, in the sense that at full turgor their dimensions and their contents are equal as well as the elastic and permeability properties of their walls and membranes.

If, on the other hand, we assume that ξ is not a function of Pc, equation 8.41 yields the following expression for the pressure dependence on the cellular volume:

$$Pc = \xi \ln(Vc/Vc_0) + Pc_0 \qquad [8.44]$$

The value of the hydrostatic pressure at full turgor, Pc_0 can be determined readily from the analysis of the differences in chemical potentials existing across the plasmalemma at that stage. Since by definition turgescence is an *equilibrium* state reached by plant cells facing an extracellular solution of *negligible* osmotic pressure (as would be achieved if the tissue was immersed in pure water [Nobel, 1983]), if we can assume for that particular condition that only *impermeable* solutes are present in the system, equilibrium will be attained when the chemical potential of *water* (subscript 1) on both sides of the barrier is equal, that is:

$$\mathcal{R}T\ln a c_{10} + \tilde{v}_1 Pc_0 = \mathcal{R}T\ln a i_{10} + \tilde{v}_1 Pi_0 \qquad [8.45]$$

where ac_{10} and ai_{10} are, respectively, the intra- and extracellular water activities at equilibrium (subscript 0) and Pi_0 is the hydrostatic pressure (in excess of the atmospheric pressure) in the interstitium.

Since it is probably reasonable to expect that the hydrostatic pressure outside the cell is always atmospheric pressure (the extracellular continuum is indeed in direct contact with an osmotic solution open to the atmosphere), equation 8.45 becomes, since $Pi_o = 0$ by definition:

$$Pc_o = -(\mathcal{R}T/\tilde{v}_1)\Delta\ln am_{1o} \qquad [8.46]$$

where $\Delta\ln am_{1o} = \ln ac_{1o} - \ln ai_{1o}$. This relation is in turn approximated, as for equation 8.38, by:

$$Pc_o \cong \mathcal{R}T\ \Sigma_k(cc_{ko} - ci_{ko}) \qquad [8.47]$$

where the summation runs for all the *impermeable* solutes present in the intra- and extracellular solutions at full turgor.

The difference in hydrostatic pressure across the plasmalemma, ΔPm, is therefore given by:

$$\Delta Pm = Pc - Pi = Pc \qquad [8.48]$$

since $Pi = 0$. Thus, as can be seen, ΔPm is evaluated directly from equations 8.43 or 8.44, in conjunction with equation 8.47. It is important to note that, since there cannot be any negative pressure present in the system, ΔPm will exist up to incipient plasmolysis, i.e., when Pc reaches 0, after which the hydrostatic pressure gradient vanishes.

From the above analysis, it then follows that the thermodynamic force involved in the *transmembrane transport* of species i, $\Delta\mu m_i$, defined formally as:

$$\Delta\mu m_i = \mathcal{R}T\Delta\ln \mathcal{a}m_i + \tilde{v}_i\Delta Pm \qquad [8.49]$$

is approximated, in the case of the solvent (i = 1), by equation 8.38 for the first term and equations 8.43 and 8.44, along with equation 8.47, for the second term. Similarly, for i = 2,3,...,m, equations 8.39 and 8.40 are used to evaluate the difference in molecular potential whereas equations 8.43 (8.44) and 8.47 are used for the difference in hydrostatic pressure.

Let us point out that, in the calculation of the above gradients, the i-th *solute* difference in chemical potential, i.e., $\Delta\mu m_i$ for i = 2,3,...,m, is evaluated only if that species is at least present on one side of the barrier. This is critical since the absence of the i-th solute on both sides of the membrane implies that it does not exist thermodynamically and, therefore, cannot be part of the estimation of the local forces. Note that this constraint also applies to the calculation of the thermodynamic forces involved in *symplastic transport*, which, as we will now see, follows basically the same approach.

In defining the thermodynamic forces pertaining to symplastic transport, one needs to consider the j-th cell and its *neighbors* in the derivation since, as we have discussed previously, this process corresponds to mass transfer between two adjacent cellular volumes. Let us recall that by virtue of the chosen *cubic* arrangement, the j-th ECUC has, in the hypothetical tissue, 6 neighboring units with which it is connected by plasmodesmata (Figure 8.5).

The number of adjoining cells which have to be accounted for in describing the above transport is nevertheless restricted to two. Since the hypothetical tissue is assimilated to a bundle of *identical* columns, i.e., that their behavior is supposed to be the same at any time, one can observe, referring to Figure 8.5, that, due to the chosen arrangement, the cellular units which are in a plane *perpendicular* to the axis of each of the ECUC's composing the representative column are also identical. Thus, since there cannot be any gradients between their intracellular solutions, we cannot expect any transfer in that planar direction. Symplastic transport is therefore restricted to movement between the j-th cell and its "downstream" and "upstream" neighbors, that is, referring to Figure 8.6, the (j-1)-th and (j+1)-th cells, respectively. Note that the cell position index *increases* from surface to interior.

Hence, the thermodynamic force involved in the *symplastic transport* of the i-th species, defined formally (as for transmembrane transport, equation 8.49) as:

$$\Delta\mu p_i^- = \mathcal{R}T\Delta \ln \mathit{a}p_i^- + \tilde{v}_i \Delta Pp^- \qquad [8.50]$$

where the superscript - indicates definitions related to the (j,j-1)-th cell interaction, will be approximated as follows:

1. For the solvent water ($i = 1$):

$$\mathcal{R}T\Delta\ln ap_1^- \cong -\mathcal{R}T\,\tilde{v}_1\,\Sigma_i(cc_i^{\,j} - cc_i^{\,j-1}) \qquad i = 2,3,\ldots,m \qquad [8.51]$$

and

$$\tilde{v}_1\Delta Pp^- \cong \tilde{v}_1(Pc^{\,j} - Pc^{\,j-1}) \qquad [8.52]$$

where $Pc^{\,j}$ and $Pc^{\,j-1}$ are evaluated using equations 8.43 (8.44) and 8.47.

2. For all other species but water:

$$\mathcal{R}T\Delta\ln ap_i^- \cong \mathcal{R}T\,(cc_i^{\,j} - cc_i^{\,j-1}) / \bar{c}p_i^- \qquad i = 2,3,\ldots,m \qquad [8.53]$$

where the average concentration across the plasmodesmata linking the j-th and (j-1)-th cell, $\bar{c}p_i^-$, is given by:

$$\bar{c}p_i^- = .5\,(cc_i^{\,j} + cc_i^{\,j-1}) \qquad [8.54]$$

The hydrostatic pressure difference, ΔPp_i^-, being the same, is evaluated using relation 8.52 where $\tilde{v}_1$ is replaced by $\tilde{v}_i$. The definitions describing the (j,j+1)-th cell interaction are obtained readily for their part simply by replacing the superscript - by + in all the preceding relations.

The choice of defining the difference operator, Δ, as the difference between *intracellular* and *extracellular* quantities— should now become clear. It is based on our desire to define the forces with respect to the j-th cell. The term "extracellular" in this context thus encompasses not only the interstitium environment (as for the differences in equation 8.38 and following), but also the cellular volumes of the neighboring cells (as for the differences in equations 8.51, 8.52 and 8.53).

The macroscopic phenomenological permeability coefficients and the flux equations

The permeability properties of the membranes involved in transmembrane and symplastic transport will be characterized by macroscopic coefficients similar to the parameters introduced in Chapter 3. We will however adopt the temperature dependent form of the coefficients since we are interested in studying *isothermal* mass transport (see equations 3.5 and 3.6).

Hence, assuming that the composition dependences disclosed in the aforementioned section are relevant (see equations 3.2 and 3.3), the macroscopic phenomenological permeability (Onsager form) of the *plasmalemma* of the j-th ECUC to the i-th species, Lm_{ii}, and to the ik-th couple, Lm_{ik} $(i,k = 1,2,...m)$, will be given by:

$$Lm_{ii} = \text{þ}m_{ii}\ \bar{c}m_{i} \qquad [8.55]$$

and

$$Lm_{ik} = \text{þ}m_{ik} \sqrt{(\bar{c}m_{i}\ \bar{c}m_{k})}\ ;\ i \neq k \qquad [8.56]$$

whereas, for the *plasmodesmata* separating the j-th cell and its neighbors, the phenomenological coefficients will be defined as:

$$Lp_{ii}^{-(+)} = \text{Þ}p_{ii}\;\bar{c}p_i^{-(+)} \qquad [8.57]$$

and

$$Lp_{ik}^{-(+)} = \text{Þ}p_{ik}\;\surd(\bar{c}p_i^{-(+)}\;\bar{c}p_k^{-(+)})$$
$$i \neq k \qquad [8.58]$$

where $\text{Þ}m_{i\,i(k)}$ and $\text{Þ}p_{i\,i(k)}$ are composition independent macroscopic permeability coefficients (assumed to apply identically to all cells), and $\bar{c}m_{i(k)}$ and $\bar{c}p_{i(k)}^{-(+)}$ are the average concentrations defined by equations 8.40 and 8.54.

It then follows that, according to the theory of irreversible thermodynamics and by analogy to the system studied in section 2.2.3, the *fluxes* of matter in or out of the j-th cell through transmembrane or symplastic transport processes (assuming passive transport only), will be evaluated from the following two basic relations (see equation 2.13); that is, for *transmembrane* flux of the i-th species, Jm_i:

$$Jm_i = -\Sigma_k Lm_{ki}\;\Delta\mu m_k$$
$$i,k = 1,2,\ldots,m \qquad [8.59]$$

and, for *symplastic* transfer, $Jp_i^{-(+)}$:

$$Jp_i^{-(+)} = -\Sigma_k Lp_{ki}^{-(+)}\;\Delta\mu p_k^{-(+)}$$
$$i,k = 1,2,\ldots,m \qquad [8.60]$$

Note that, due to the definition of the difference operator, Δ, the minus sign in front of the above equations is necessary to stay in agreement with the convention adopted in section 2.2.3 that an *efflux* is *negative*, i.e. equivalent to a *loss* and an *influx* is *positive*, i.e. equivalent to a *gain*.

8.2.2 Interstitium equations of continuity

The development of the interstitium equations of continuity, i.e. the relations describing the transient behavior of the species diffusing in the extracellular volume, is based, in part, on the work of Weinstein and Stephenson (1979) on transport of electrolytes across a simple monolayer epithelium. The close structural analogy existing between the specialized animal tissue and our hypothetical cylindrical cell (the reader is referred to the above reference for more details) indeed makes the mathematical description of both systems quasi-identical, which turns out to be particularly valuable, especially in view that the model proposed by the above authors considers the interaction of the epithelium cell with its extracellular environment through the use of relations also derived from the theory of irreversible thermodynamics.

Thus, referring to Figure 8.6, let us consider the j-th ECUC, more precisely its interstitium cylinder. In developing the equations describing the conditions associated with mass transport in this extracellular volume, the following assumptions will be used:

1. Axial diffusion occurs in the interstitium cylinder.
2. Radial and azimuthal diffusion are rapid in comparison to axial diffusion.
3. The interstitial volume is allowed to vary (see section 8.1.2) as well as the cross-sectional area of transfer. However, since the cells are treated as *whole entities* as to their response to volume changes, the transfer of cellular volume to interstitial volume (stage 2) is assumed to be spread *equally* over the entire interstitium, which therefore forces the mensural rearrangements to be performed on the *whole structure*. This means that the radii of the cylinders composing the ECUC, its length and its other geometrical characteristics will be functions of *time* and *distance* in the column, but on a *cell-to-cell basis*. We can therefore anticipate a *stepwise* behavior from the column constituents as to the variation in their mensurations.
4. The macroscopic permeability coefficients (þ form) describing the properties of the plasmalemma are constant in time and position along the interstitium and are assumed identical for all the cells.
5. The description is restricted to *nonelectrolyte* transport. There are therefore no Donnan exchange or any other form of ionic interactions present in the system.
6. The extracellular solution is, at all times, sufficiently dilute to make the interactions between diffusing species negligible.

After the presentation of these governing assumptions, let us now restrict the discussion to considering a representative slice of the j-th interstitium cylinder, i.e., let us define a *differential volume element* of cross-sectional area Ai and thickness $\delta\ell$, where δ is a small fraction of the total cell length constant with time (see Figure 8.6). Applying the law of *conservation of mass* to the above differential element, one can write a *molar (mass) balance* for the i-th species diffusing in this volume. The various contributions to the balance are:

1. Input of component i across the face at z:

$$Ni_j|z\ [Vi(avg)/\ell(avg)]\ \Delta\theta \qquad [8.61]$$

where Ni_j is the *molar flux* of the i-th species in the j-th interstitium with respect to a *fixed* frame of reference chosen as the *center* of the infinite slab when this geometry is considered, or as a point sufficiently distant in the tissue to be unaffected by the mass transport processes, when transfer in a semi-infinite medium is studied. The notation |z means "evaluated at z". Vi(avg) and ℓ(avg) are, respectively, *average* values of the interstitial volume and the ECUC length for the time *interval* $\Delta\theta$ (see assumption 3 above). Note that the symbol Δ is used here to indicate an interval of a given *continuous* variable. The reader need not however be concerned with possible confusion with the *difference operator*, Δ, used previously, since the former symbol vanishes during the differentiation process (see below).

2. Output of component i across the face at $z+\Delta z$:

$$Ni_{j}|_{z+\Delta z}\ [Vi(avg)/\ell(avg)]\ \Delta\theta \qquad [8.62]$$

where Δz is the thickness of the differential element given by $\delta\ell$.

3. Diffusional flow through the plasmalemma:

$$Jm_{j}\ [2\pi\ Ri(avg)\ \delta\ell(avg)]\ \Delta\theta \qquad [8.63]$$

4. Time change of the molar content of species i in the volume element:

$$[(ci_{j}\ Ai\ \ell)|_{\theta+\Delta\theta} - (ci_{j}\ Ai\ \ell)|_{\theta}]\delta \qquad [8.64]$$

When the entire molar balance is written down and divided throughout by δ and $\Delta\theta$, one obtains, after letting the size of the intervals Δz and $\Delta\theta$ decrease to zero:

$$\partial(ci_{j}\ Vi)/\partial\theta + Vi\ \partial Ni_{j}/\partial z + 2\pi\ Ri\ \ell\ Jm_{j} = 0 \qquad [8.65]$$

This is the *equation of continuity* for component i diffusing in the j-th interstitium. Similar relations can be written for all diffusing species, i.e., $i = 1,2,...m$. However only $m-1$ relations are independent. The last term of equation 8.65 will be referred to as the *production-depletion term* since it describes how transmembrane exchanges affect the local composition of the extracellular solution. This is by analogy with the *reaction term* found in equations of continuity detailing mass transport in the presence of chemical reactions (Bird *et al.*, 1960).

Further insight can be gained if we apply the rule of differentiation of a product to the first term of equation 8.65, such that the relation becomes:

$$\partial ci_i/\partial\theta =$$

$$-[\partial Ni_i/\partial z + Am\ Jm_i/Vi + (ci_i/Vi)\partial Vi/\partial\theta] \qquad [8.66]$$

where, by definition (see equation 8.24), $Am = 2\pi\ Ri\ \ell$. As can be seen, the time variation of the concentration of species i in the interstitium is explicitly a function of three processes: transport of molecules by diffusional forces, production or depletion due to transmembrane exchanges, and "concentration" or "dilution" due to interstitial volume changes. Note that, in the case of an *impermeable* component, the production-depletion term vanishes since the species cannot cross the barrier in either way. It should be emphasized here, as discussed in section 8.1.2, that the extracellular diffusion phenomenon, i.e. the *apoplastic* transport, is described exclusively with respect to the interstitial volume. One can observe that, as far as the mathematical "picture" is concerned, the cellular and buffer cylinders do not appear in the derivation.

The equation relating the fluxes of all the components present in the system can be developed for its part by consideration of the *volume* of the interstitium cylinder. Making a *"volumetric balance"* on the differential element defined earlier[16], one gets the following contributions:

1. Volume inflow at z:

$$\Sigma_i \tilde{v}_i Ni_i|z \; [Vi(avg)/\ell(avg)] \; \Delta\theta \qquad i = 1,2,\ldots,m \qquad [8.67]$$

2. Volume outflow at z+Δz:

$$\Sigma_i \tilde{v}_i Ni_i|z{+}\Delta z \; [Vi(avg)/\ell(avg)] \; \Delta\theta \qquad i = 1,2,\ldots,m \qquad [8.68]$$

3. Volume flow through plasmalemma:

$$\Sigma_i \tilde{v}_i Jm_i \; [2\pi \; Ri(avg) \; \delta\ell(avg)] \; \Delta\theta \qquad i = 1,2,\ldots,m \qquad [8.69]$$

4. Volume accumulation in differential element:

$$[(Ai \; \ell)|\theta{+}\Delta\theta - (Ai \; \ell)|\theta]\delta \qquad [8.70]$$

leading, after a process similar to the one used to generate equation 8.65, to the *total equation of continuity* for the present system, i.e.:

$$\partial Vi/\partial\theta + Vi \; \partial(\Sigma_i \tilde{v}_i Ni_i)/\partial z + Am \; \Sigma_i \tilde{v}_i Jm_i = 0 \qquad i = 1,2,\ldots,m \qquad [8.71]$$

---

[16] Strictly speaking, the term "*volumetric balance*" is inappropriate since only *mass* and *energy* are truly conserved. However, if one assumes that the partial molar volumes of the diffusing species are constant, i.e. there are no volume changes due to interactions between the diffusing molecules (see assumption 6), it then becomes possible to apply a "conservation" law to that quantity.

which, since by definition $\Sigma_j \tilde{v}_j Ni_j = v^{\blacksquare}$, known as the *volume average velocity* (Bird *et al.*, 1960; Clazie *et al.*, 1967), can be rewritten as:

$$\partial v^{\blacksquare}/\partial z = -[(Am/Vi)\Sigma_j \tilde{v}_j Jm_j + (1/Vi)\partial Vi/\partial \theta] \qquad i = 1,2,\ldots,m \qquad [8.72]$$

Still, the continuity equations defined in relations 8.66 and 8.72 are not in useful form for obtaining *concentration profiles*. In order to get the equations generally used for describing diffusion processes, we replace the fluxes Ni_j by the appropriate expressions involving concentration gradients. Hence, we can make use of a *generalized* form of Fick's law for *multicomponent* diffusion, that is, for the i-th species diffusing in the j-th interstitial volume (see Clazie *et al.*, 1967):

$$Ni_j = Ji^{\blacksquare}_j + ci_j\, v^{\blacksquare} \qquad [8.73]$$

and

$$Ji^{\blacksquare}_j = -\Sigma_k \text{đ}^{\blacksquare}_{kj}\, \partial ci_k/\partial z \qquad k = 1,2,\ldots,m-1 \qquad [8.74]$$

where $Ji^{\blacksquare}_j$ is the *diffusive molar flux* expressed with respect to the volume average velocity, $v^{\blacksquare}$, and $\text{đ}^{\blacksquare}_{kj}$ is a *generalized multicomponent diffusivity* not identifiable in general with binary diffusion coefficients (Chandrasekaran and King, 1972; Miller, 1960). The summation in equation 8.74 runs for *m-1* components since the diffusive fluxes are linked through the following relation:

$$\Sigma_i \tilde{v}_i Ji^{\blacksquare}_i = 0 \ ; \ i = 1,2,\ldots,m \qquad [8.75]$$

leaving only *m-1* independent fluxes. Equation 8.74 is similar to equations 8.59 and 8.60 in the sense that it describes how the flux of the i-th component is a function not only of its own concentration gradient but also of the gradients of the other species present in the interstitium.

In its present form, the complete system of continuity equations obtained by inserting equations 8.73 and 8.74 in relation 8.66 is barely manageable. One can however reduce the complexity of the preceding relations by supposing that the extracellular solution is sufficiently dilute to make the interactions between the diffusing species negligible (assumption 6), such that equation 8.74 becomes:

$$Ji^{\blacksquare}_i = -Đ^{\blacksquare}_i \ \partial ci_i/\partial z \qquad [8.76]$$

where $Đ^{\blacksquare}_i$ is a *pseudo-binary* solution diffusion coefficient (Bird *et al.*, 1960) which will be assumed to be identical to the *binary* diffusivity of the i-th species in water, $D^{\blacksquare}_i$. Note that the symbol ■ supposes that the diffusion coefficients to be used are based on measurements obtained by employing the volume average velocity of the studied systems as reference (Crank, 1975). Equation 8.66 can then be rewritten as:

$$\partial ci_i/\partial\theta =$$

$$-[\partial/\partial z(-Đ^{\blacksquare}_i \ \partial ci_i/\partial z + ci_i \ v^{\blacksquare}) +$$

$$\mathrm{Am}\ Jm_{i}/Vi + (ci_{i}/Vi)\partial Vi/\partial\theta] \qquad [8.77]$$

leading to, after applying the rule of differentiation of a product to the first term and noting that the diffusion coefficient, $\text{Đ}^{\blacksquare}_{i}$, is kept concentration dependent:

$$\partial ci_{i}/\partial\theta =$$

$$\partial/\partial z(\text{Ƀ}^{\blacksquare}_{i}\ \partial ci_{i}/\partial z) - v^{\blacksquare}\ \partial ci_{i}/\partial z -$$

$$[\partial v^{\blacksquare}/\partial z + (1/Vi)\partial Vi/\partial\theta]ci_{i} - \mathrm{Am}\ Jm_{i}/Vi \qquad [8.78]$$

where $\text{Ƀ}^{\blacksquare}_{i}$ is the pseudo-binary diffusivity of species i *corrected* to take into account the hindrance effect of the interstitium microstructure (see section 8.1.1), that is:

$$\text{Ƀ}^{\blacksquare}_{i} = \psi i\ \text{Đ}^{\blacksquare}_{i} = \psi i \cdot D^{\blacksquare}_{i} \qquad [8.79]$$

It should be emphasized, before turning our attention to the derivation of the intracellular equations of change, that relations 8.72 and 8.78 are, by virtue of the governing assumptions and the nature of the extracellular volume, *point functions in time and distance* along the interstitium. However, since the geometrical properties of the ECUC composing the column vary from cell to cell in response to the volumetric rearrangements, these equations are confined to the interstitium of the cell under consideration. Proper boundary conditions are therefore required to link the individual descriptions of interstitial mass transfer, to lead to a global picture of the diffusion in the entire apoplastic continuum (see section 8.2.5).

8.2.3 Intracellular equations of change

In the derivation of the equations describing the volume and concentration changes in the intracellular volume, the following assumptions will be considered:

1. No radial, azimuthal or axial diffusion occurs in the cellular volume. Perfect mixing is assumed.
2. The plasmalemma surface area, Am, is allowed to vary (see section 8.1.2) as is the total symplastic area of transfer, Ap, since both are linked through the factor αp. Indeed, referring to equations 8.10 and 8.23, the latter area is the following explicit function of the former; that is, since $Ap' = \alpha p\ Ac'$ (equation 8.10) and $Am' = (1-\alpha p)\ Ac'$ (equation 8.23):

$$Ap = [\alpha p/(1-\alpha p)]\ Am \qquad [8.80]$$

where the superscript 1 has been dropped to indicate properties of the j-th ECUC.

3. The macroscopic permeability coefficients (Þ form) describing the properties of the plasmodesma pseudo-membrane are constant in time and are assumed to apply identically for all the cells.

Hence, referring to Figure 8.6, one ~~can write~~, for the j-th cellular volume considered as a *whole*, the following contributions to the variation of the *molar concentration* of the i-th species:

1. Inflow/outflow from/to the interstitium[1]:

$$2\pi\ Ri(avg) \int Jm_i dz\ \Delta\theta \qquad [8.81]$$

where the integral is carried out over the entire length of the cell, $\ell(avg)$, i.e. the average length for the time interval $\Delta\theta$.

2. Inflow/outflow from/to the (j-1)-th cell:

$$Ap^{-}(avg)\ Jp_i^{-}\ \Delta\theta \qquad [8.82]$$

where $Ap^{-}(avg)$ is the average value, for the time interval $\Delta\theta$, of the *fraction* of *total symplastic surface area* pertaining to the (j,j-1)-th cell interface, i.e.:

$$Ap^{-}(avg) = \min[Ap^{j}(avg), Ap^{j-1}(avg)]/6 \qquad [8.83]$$

The denominator supposes that the total symplastic surface area of any given cell can be divided into 6 equal parts corresponding to the fraction of plasmodesmata abutting on each of the 6 neighboring cells (see Figure 8.5). The numerator, for its part, is a direct outcome of assumption 2, which forces one to choose the smallest transfer area linking the 2 cells since their plasmalemma might have different surface areas. Note that the symbol (avg) drops after the differentiation process (see below).

3. Inflow/outflow from/to the (j+1)-th cell:

---

[1] Note that the direction of flow is determined by the sign taken by the fluxes, i.e., positive if mass is gained by the cell and negative if mass is lost (see section 8.2.1).

$$Ap^{+}(avg)\ Jp_{i}^{+}\ \Delta\theta \qquad [8.84]$$

where, as for equation 8.83, $Ap^{+}(avg)$ is given by:

$$Ap^{+}(avg) = \min[Ap^{j}(avg),\ Ap^{j+1}(avg)]/6 \qquad [8.85]$$

4. Time change of the molar content of species i in the cellular volume:

$$[(cc_{i}\ Vc)|\theta+\Delta\theta - (cc_{i}\ Vc)|\theta] \qquad [8.86]$$

After writing down the entire molar balance, dividing throughout by $\Delta\theta$ and taking the limit as $\Delta\theta$ tends to zero, one obtains:

$$d(cc_{i}\ Vc)/d\theta = $$
$$2\pi\ Ri\ \int Jm_{i} dz + Ap^{-}\ Jp_{i}^{-} + Ap^{+}\ Jp_{i}^{+} \qquad [8.87]$$

which, by applying the rule of differentiation of a product on the left-hand side, leads finally to:

$$dcc_{i}/d\theta = $$
$$(2\pi\ Ri/Vc)\int Jm_{i} dz - (cc_{i}/Vc) dVc/d\theta + $$
$$(Ap^{-}/Vc)Jp_{i}^{-} + (Ap^{+}/Vc)Jp_{i}^{+} \qquad [8.88]$$

Relation 8.88 is entirely analogous to equation 4.2, where the first term describes the contribution of the transmembrane fluxes and the second the effect of the cellular volume change on the concentration variation. The last two terms are however peculiar to the present treatment since they describe the contribution of symplastic transport, which was inexistent in the context of the derivation of relation 4.2. Note also that the integral sign is a consequence of the *local nature* of equations 8.59 (see section 8.2.1) and 8.78. Due to the presence of concentration gradients in the interstitium, the local transmembrane fluxes vary from one end to the other. They must therefore be integrated over the entire length of the cell to obtain the global contribution to the cellular volume.

By a treatment similar to the preceding one, we can derive the *total volume variation* of the cellular compartment, $dVc/d\theta$, which, analogously to equation 4.1, is given by:

$$dVc/d\theta = 2\pi\ Ri \int \Sigma_i \tilde{v}_i Jm_i\, dz + Ap^- \Sigma_i \tilde{v}_i Jp_i^- + Ap^+ \Sigma_i \tilde{v}_i Jp_i^+ \qquad i = 1,2,\ldots,m \qquad [8.89]$$

where, again, we can see the contributions of the transmembrane and symplastic fluxes to the total volume change.

In the derivation of equation 8.88 it was assumed that the cellular volume, Vc, was identical to the *intracellular solution* or *osmotic* volume, Vo, that is, the volume containing the material which can *effectively* participate in the transmembrane and symplastic exchanges. However, as discussed in section 4.1.1, the estimation of the thermodynamic forces relies on the assumption that both the intracellular and extracellular solutions are *ideal* and *dilute*. Hence, referring to the aforementioned section, we will use the concept of the *apparent cellular nonosmotic volume*, Vd, to account for the intrinsic nonideality and nondiluteness of the intracellular solution such that the above assumption is valid. This nonosmotic volume will be supposed constant with time and assumed to occupy the same proportion of total cellular volume for all the cells. Equation 8.88 then becomes:

$$dc c_i/d\theta = [2\pi Ri/(Vc-Vd)]\int Jm_i\,dz - [cc_i/(Vc-Vd)]\,dVc/d\theta + [Ap^-/(Vc-Vd)]Jp_i^- + [Ap^+/(Vc-Vd)]Jp_i^+ \qquad [8.90]$$

where, by definition, Vo = Vc - Vd (see equation 4.8).

8.2.4 Supplementary equations of change

Geometrical relations

In the development of the model, geometrical relations were used to evaluate the equivalent cylindrical unit cell mensurations (see section 8.1.2). The description of the evolution of these geometrical properties as time passes becomes crucial since these parameters are intrinsically part of the continuity equations presented in the preceding sections. These mensural relations, by virtue of their nature and of the assumptions adopted previously, represent the geometrical characteristics of *entire cellular units*. They are therefore independent of position *within* a given ECUC. The geometrical equations of change then become *time* derivatives which, for the j-th unit cell, are:

1. The time variation of unit length, $d\ell/d\theta$. Referring to equations 8.17 and 8.18, the length of the j-th ECUC is the following function of the *total* unit volume, V:

$$\ell = (6\ V/\pi)^{1/3} \sqrt{\tau i} \qquad [8.91]$$

Taking the derivative with respect to time yields:

$$d\ell/d\theta = [(\sqrt{\tau i})/3\ (6/\pi)^{1/3}]\ V^{-2/3}\ dV/d\theta \qquad [8.92]$$

2. The time variation of interstitium cylinder radius, $d\mathrm{Ri}/d\theta$. From equation 8.25, one obtains, after differentiation:

$$d\mathrm{Ri}/d\theta = (1/2\pi)(\ell^{-1}\ d\mathrm{Am}/d\theta - \mathrm{Am}\ \ell^{-2}\ d\ell/d\theta) \qquad [8.93]$$

3. The time variation of cellular and buffer cylinder radii. Referring to equations 8.27 and 8.29, one generates, respectively:

$$d\mathrm{Rc}/d\theta = [1/(2\pi\ \mathrm{Rc})](\ell^{-1}\ d\mathrm{Vc}/d\theta - \mathrm{Vc}\ \ell^{-2}\ d\ell/d\theta) + (\mathrm{Ri}/\mathrm{Rc})\ d\mathrm{Ri}/d\theta \qquad [8.94]$$

and

$$d\mathrm{Rb}/d\theta = (\mathrm{Ri}/\mathrm{Rb})\ d\mathrm{Ri}/d\theta - [1/(2\pi\ \mathrm{Rb})](\ell^{-1}\ d\mathrm{Vi}/d\theta - \mathrm{Vi}\ \ell^{-2}\ d\ell/d\theta) \qquad [8.95]$$

4. The time variation of the apparent interstitium diffusibility, $d\psi i/d\theta$. By definition (equation 8.9), the

overall resistance factor of the interstitium of the j-th cell is given by:

$$\psi i = [\phi s + (\phi w \ \psi w)]/\epsilon \qquad [8.96]$$

where the superscript 1 has been dropped and $\epsilon = \phi s + \phi w$ (see equation 8.1). Equation 8.96 can thus be rewritten as:

$$\psi i = [\epsilon - (1 - \psi w)\phi w]/\epsilon \qquad [8.97]$$

leading to:

$$\psi i = 1 - [(1 - \psi w)Vw/Vi] \qquad [8.98]$$

since, by definition, $\epsilon = Vi/V$ (equation 8.1) and $\phi w = Vw/V$ (equation 8.2), where Vw is the *cell wall volume* pertaining to the j-th ECUC.

Taking the derivative of the above relation with respect to time yields:

$$d\psi i/d\theta = -(1 - \psi w)(Vi^{-1} \, dVw/d\theta - Vw \, Vi^{-2} \, dVi/d\theta) \qquad [8.99]$$

If we now assume that any increase (decrease) in interstitium volume (when it is allowed; see below) corresponds to a gain (loss) of *intercellular free space volume* only (see section 8.1.1), that is, we suppose that the *cell wall volume* neither expands nor shrinks, equation 8.99 becomes:

$$d\psi i/d\theta = (1 - \psi w)(Vw/Vi^2) \, dVi/d\theta \qquad [8.100]$$

since, from the above assumption, $dVw/d\theta = 0$.

5. The time variation of the apoplastic transfer area, $dAi/d\theta$. From equation 8.35, one obtains similarly:

$$dAi/d\theta = 2\pi(Ri\ dRi/d\theta - Rb\ dRb/d\theta) \qquad [8.101]$$

Changes during dehydration

Since the behavior of a typical cell undergoing osmotic dehydration has been discussed in detail (see section 8.1.1), we will confine ourselves here to presenting the general equations describing internal volume and transfer area rearrangements. Thus, referring to equations 8.14 and 8.15, the interstitium and total volume changes are related to the cellular volume variation as follows (for the j-th ECUC):

$$dVi/d\theta = \kappa\ dVc/d\theta \qquad [8.102]$$

and

$$dV/d\theta = (1 + \kappa)\ dVc/d\theta \qquad [8.103]$$

where the compliance factor, κ, takes the values of 0, -1 and 0, for stages 1, 2 and 3, respectively. The variations in plasmalemma and total symplastic surface area are given for their part by:

1. For stage 1, referring to equation 8.11:

$$dAm/d\theta = [(2\ Am_0)/(3\ Vc_0^{2/3})]\ Vc^{-1/3}\ dVc/d\theta \qquad [8.104]$$

where Am_0 and Vc_0 are reference values. This leads therefore to a proportional change in symplastic transfer area since, from equation 8.80:

$$dAp/d\theta = [\alpha p/(1-\alpha p)]\, dAm/d\theta \qquad [8.105]$$

2. For stages 2 and 3, constancy is assumed for the above surface areas; it then follows that $dAm/d\theta = 0$ and, accordingly, $dAp/d\theta = 0$.

Overall mass variation

In the study of mass transport phenomena in a tissue, it is also interesting to look at the *total mass* variation as well as the *net intake* (or *loss*) of certain solutes present in the system. The next relations are therefore derived to evaluate these changes. They are based on the following assumptions:

1. The shape and size of the studied piece of tissue are such that they can be assimilated either to a semi-infinite medium or to an infinite slab. This is important if one wants to compare experimental to predicted data.
2. It is assumed that there are no "*cut*" cells on the surface(s) of the tissue exposed to the bathing solution. To take into account this possibility (in comparing with experimental data), the "thickness" of the piece (which determines, as we will see, the number of ECUC in a column [see section 8.2.5]) should be reduced proportionally to the percentage of cut cells on the surface(s) since the model considers the cells as *complete entities*.

Hence, writing a *total mass balance* on a piece of tissue of finite mass, m', and remembering that the total mass variation can be described ultimately with respect to exchanges occurring at the surface(s) of the tissue in contact with the osmoticum (symbol "|."), one obtains:

$$
\begin{aligned}
&dm'/d\theta = \\
&\quad -(\#col')(Ai\ \Sigma_i M_i Ni_i - Ap^-\ \Sigma_i M_i Jp_i^-)|_{.} \\
&\quad i = 1,2,\ldots,m
\end{aligned}
\tag{8.106}
$$

where #col' is the *total number of columns* representing the tissue under consideration and M_i is the molecular weight of species i. As can be seen, the total mass variation of the tissue is a function of the mass gained or lost through the apoplast (first term) and the symplast (second term) of the representative column, which, by virtue of its design, is open to the osmotic bath at the level of the surface cell. Note that the minus sign is a direct consequence of the choice of a fixed frame of reference for the molar fluxes, Ni_i, attached to the center or to a distant point within the tissue. The fluxes are therefore positive if they are directed toward the surface and negative if the species move in the opposite direction (see section 8.2.2).

The net intake or loss of species i can be derived similarly, leading to:

$$
\begin{aligned}
&dm_i/d\theta = \\
&\quad -(\#col')(Ai\ M_i Ni_i - Ap^-\ M_i Jp_i^-)|_{.}
\end{aligned}
\tag{8.107}
$$

Note however that only m-1 of such equations are independent, since by definition:

$$dm'/d\theta = \Sigma_i dm_i/d\theta \;;\; i = 1,2,\ldots,m \qquad [8.108]$$

Equation 8.106 can be made an exclusive function of the *solute* fluxes by employing the definition of the volume average velocity, $v^{\blacksquare}$. Referring to section 8.2.2, $v^{\blacksquare}$ is given by:

$$v^{\blacksquare} = \tilde{v}_1 Ni_1 + \Sigma_i \tilde{v}_i Ni_i \;;\; i = 2,3,\ldots,m \qquad [8.109]$$

which leads to:

$$Ni_1 = (v^{\blacksquare} - \Sigma_i \tilde{v}_i Ni_i)/\tilde{v}_1 \;;\; i = 2,3,\ldots,m \qquad [8.110]$$

where, as before, the subscript 1 refers to the solvent water. Inserting this relation in the first term of equation 8.106 and after some rearrangement, one obtains:

$$\begin{aligned} dm'/d\theta = {} & \\ & -(\#col')\, Ai|_s\, (M_1/\tilde{v}_1)[v^{\blacksquare} - \Sigma_i \tilde{v}_i Ni_i + \\ & (\tilde{v}_1/M_1)\Sigma_i M_i Ni_i]|_s + (\#col')(Ap^- \,\Sigma_i M_i Jp_i^-)|_s \\ & i = 2,3,\ldots,m \end{aligned} \qquad [8.111]$$

which becomes, after regrouping the terms under the same summation sign:

$$\begin{aligned} dm'/d\theta = {} & \\ & -(\#col')\, Ai|_s\, (M_1/\tilde{v}_1)\{v^{\blacksquare} - \Sigma_i [\tilde{v}_i - (\tilde{v}_1 M_i/M_1)] Ni_i\}|_s \\ & + (\#col')(Ap^- \,\Sigma_i M_i Jp_i^-)|_s \;;\; i = 2,3,\ldots,m \end{aligned} \qquad [8.112]$$

8.2.5 Initial and boundary conditions

The equations of continuity presented in sections 8.2.2 and 8.2.3 have a family of solutions, which are dependent on the *initial* and *boundary conditions* employed to solve the system. One needs therefore to define carefully these conditions if proper simulations are to be obtained. This is what will be discussed in the next two sections.

Initial conditions

We will assume that initially the tissue is at *full turgor* (subscript 0), that is, it has been equilibrated with pure water. An average unit cell can then be generated (see section 8.1.1) from the analysis of the geometrical characteristics of the tissue in that state, i.e. estimation of the average diameter of the cells composing the tissue, dc'_0; evaluation of the fraction of the total volume occupied by intercellular free space and cell wall volumes, $\phi s'_0$ and $\phi w'_0$; and estimation of the diffusibility of the cell wall complex, ψw. This presupposes that initially all the constitutive cells are *identical*, that is, *on average* their dimensions and their contents are equal as are the elastic and permeability properties of their walls and membranes.

Two critical numbers need to be determined at that level. Firstly, the *number of cellular units in "thickness"*, #c, that is, the number of average unit cells required to go from one surface to the other, in the case of an infinite slab'', or from the surface to a point sufficiently distant in the tissue not to be affected by osmotic stresses, in the case of a semi-infinite body. Secondly, the *number of cellular "strings"* representing the tissue, #col', is calculated such that:

$$(\#c)(\#col') = \#c' = V'_o \; / \; V'_o \qquad [8.113]$$

where, as before, V'_o and V'_o are, respectively, the total volume at full turgor of the average unit cell and of the tissue under consideration, and #c' is the total number of cellular units composing the structure (see equation 8.4).

The number of units in a column of cells, #c, is given by:

$$\#c = \mathrm{int}[(T_o \; / \; d'_o) + 1] \qquad [8.114]$$

where T_o is a reference thickness taken as *half* the *corrected thickness* (see section 8.2.4) of the tissue, in the case of an infinite slab, or, for a semi-infinite medium, as a *corrected finite distance from the surface* chosen such that the conditions at any point located at T_o within the tissue remain constant and equal to the *initial*

''Note that, as discussed previously (see section 8.1.2), we assume that the tissue under consideration can be assimilated either to a semi-infinite body or to an infinite slab. It is important to realize that, although the term "infinite" is used to describe both geometries, *finite* values are necessary for calculation purposes. The terminology therefore implies that the dimensions of the tissue, particularly the ratio of surface area to thickness, are such that the finite body can be considered analogous to these infinite structures.

ones during the entire duration of the dehydration process (see next section); d'_o is the diameter of the sphere representing the average unit cell (see Figure 8.4). Note that #c is taken as the nearest *integer* greater than or equal to the calculated value since the model assumes that the cells are physically sound throughout the tissue.

Following the approach described in section 8.1.2, one then proceeds to devise an equivalent cylindrical unit cell based on the initial properties of the average unit cell. The conditions in the representative column of #c ECUCs can therefore be represented for $\theta = 0$ as follows:

1. For all #c cells, i.e. for $j = 1,2,\ldots,\#c$: $Rb^j = Rb_o$; $Ri^j = Ri_o$; $Rc^j = Rc_o$; $\ell^j = \ell_o$; $Am^j = Am_o$; $Ap^j = Ap_o$; $Ai^j = Ai_o$; $Vc^j = Vc_o$; $Vi^j = Vi_o$; $V^j = V_o$; $\psi i^j = \psi i_o$.
2. For all #c cells and all solutes present in the system, i.e. $j = 1,2,\ldots,\#c$ and $i = 1,2,\ldots,m$: $Cc_i^j = Cc_{io}$; $Ci_i^j = Ci_{io}$.
3. Since it is assumed that initially the tissue is in equilibrium with pure water, there is, by definition, no symplastic transport and no transmembrane or apoplastic fluxes across and along the entire length of all the interstitia, i.e. for $i = 1,2,\ldots,m$ and $\theta = 0$: Jp_i^-, Jp_i^+, Jm_i, and Ni_i are null and, accordingly, $v^{\blacksquare} = 0$ in all extracellular volumes.
4. Finally, the initial mass of the tissue and of the species present in it are given by: $m' = m'_o$ and $m_i = m_{io}$ for $i = 2,3,\ldots,m$.

Boundary conditions

These conditions describe the behavior of the system at certain important points *in* the tissue for $\theta > 0$, i.e. for the period during which the tissue is in contact with the osmoticum. Three locations will be considered: the surface of the tissue, the "center" and the interface between two adjoining cells.

1. Conditions at the *surface*. At any time, the *convective* mass transfer at the surface of the tissue will be given by (Geankoplis, 1978):

$$Ni_i|_s = K_i\,(ci_i|_s - cb_i) \qquad i = 1,2,\ldots,m \qquad [8.115]$$

where K_i is the *pseudo-binary mass transfer coefficient* of the i-th solute at the surface and cb_i is the bath concentration of that species. Note that it is assumed that the volume of the osmotic solution is such that its composition remains unaltered by the dehydration process, i.e. for $\theta > 0$, $cb_i = cb_i$ at $\theta = 0$.

Equation 8.115 can be rewritten to introduce the i-th concentration gradient at the surface by replacing the left-hand side by equations 8.73, 8.76 and 8.79, leading to:

$$(\partial ci_i/\partial z)|_s = \{[(v_\blacksquare - K_i)/B_{\blacksquare i}]ci_i\}|_s + (K_i/B_{\blacksquare i}|_s)cb_i \qquad i = 1,2,\ldots,m \qquad [8.116]$$

For a situation where no resistance to mass transfer exists at the surface, i.e. $K_i \rightarrow \infty$, condition 8.116 becomes equivalent, for $\theta > 0$, to:

$$Ci_i|_s = Cb_i \; ; \; i = 1,2,\ldots,m \qquad [8.117]$$

The volume average velocity at the surface, $v\blacksquare|_s$, can be obtained by considering the *total* volume change of the model column. Hence, writing a *total "volumetric balance"* (see section 8.2.2) on the column of total volume, $\Sigma_j V^j$, $j = 1,2,\ldots,\#c$, and, since all volume lost or gained must cross the surface through the apoplastic and symplastic transfer area of the first ECUC, one obtains:

$$\begin{aligned} &d(\Sigma_j V^j)/d\theta = \\ &\quad -(A_i \; \Sigma_i \tilde{v}_i Ni_i)|_s + (Ap^- \; \Sigma_i \tilde{v}_i Jp_i^-)|_s \\ &\quad i = 1,2,\ldots,m \text{ and } j = 1,2,\ldots,\#c \end{aligned} \qquad [8.118]$$

which yields, by making use of the definition of $v\blacksquare$ (see section 8.2.2):

$$\begin{aligned} &v\blacksquare|_s = \\ &\quad [(Ap^-/Ai)\Sigma_i \tilde{v}_i Jp_i^-]|_s - (1/Ai)|_s \; \Sigma_j dV^j/d\theta \\ &\quad i = 1,2,\ldots,m \text{ and } j = 1,2,\ldots,\#c \end{aligned} \qquad [8.119]$$

2. Conditions at the "*center*". Two types of conditions need to be discussed here. They are based on the characteristics of the two tissue geometries introduced previously, i.e. the semi-infinite medium and the infinite slab. In the former case, the "center" (symbol "$|_o$") will be defined as a plane parallel to the surface, situated initially at a distance T_o

within the tissue. For any points located on that plane and beyond, we will suppose that the *interstitium* and *cellular* conditions remain, at any time, equal to the *initial* conditions, that is, for $\theta > 0$:

$$ci_i|_o = ci_{io} \text{ and } cc_i|_o = cc_{io}$$
$$i = 1,2,\ldots,m \qquad [8.120]$$

In the latter geometry, the "center" is assimilated obviously to the plane parallel to the exposed surfaces, which separates the infinite slab into two equal parts. It is assumed, in this case, that both moieties behave alike. It then follows, by the requirement of *symmetry*, that *at the center*:

$$(\partial ci_i/\partial z)|_o = 0 \text{ ; } i = 1,2,\ldots,m \qquad [8.121]$$

Since, for both geometries, the center plane is located, by virtue of equation 8.114, at the "bottom" end of the last ECUC composing the representative column, the following conditions become necessary to complete the description of the behavior of the tissue *at the center*:

a. For all species there are no symplastic fluxes allowed across the interface separating the last and (last + 1) cells, i.e.:

$$Jp_i^{*}|_o = 0 \text{ for } i = 1,2,\ldots,m \qquad [8.122]$$

This condition arises from the fact that, in the case of a semi-infinite body, the last cell is supposed to be located far enough in the tissue to ensure that its cellular content and the content of the next cell are basically unaltered by the transport processes, which therefore prevents the establishment of gradients responsible for symplastic transport. Analogously, in the case of the infinite slab, the interface separates 2 cells having, by symmetry, the same properties, which again precludes any symplastic transport.

b. For similar reasons, it follows that the volume average velocity gradient at the center, i.e. $(\partial v^{\blacksquare}/\partial z)|_o$ (given by equation 8.72) and the volume average velocity at that location, $v^{\blacksquare}|_o$, are null. We will return to these last two conditions in the following discussion.

3. Conditions at the *interface* separating two adjoining cells. As mentioned in section 8.2.2, the description of the behavior of the representative column is approached on a *cell-to-cell* basis. The major consequence of such treatment is that it necessarily creates *discontinuities* at the *interface* between 2 adjoining cells since the 2 neighboring units do not behave alike. The interstitium continuity equation (equation 8.78) is consequently not valid at the interface due to the different cell properties on the 2 sides. Thus, a special analysis of mass transfer is required to link the transport phenomena occurring in each cell *at the interface.*

Hence, referring to Figure 8.6, let us consider a differential element lying on the interface separating the j-th and (j+1)-th ECUC. The size of this differential volume is chosen small enough to ensure that there are neither transmembrane fluxes nor accumulation of material in the element[19]. Writing a *molar balance* on the element and taking the limit as its size tends to zero leads to:

$$(Ai^{j}\ Ni_{i}^{j})|_{in} = (Ai^{j+1}\ Ni_{i}^{j+1})|_{in}$$

$$i = 1,2,\ldots,m \qquad [8.123]$$

Equation 8.123 simply states that *at the interface* (symbol "$|_{in}$") there is conservation of mass for all species. The superscripts j and (j+1) indicate that the quantities are characteristics of the j-th and (j+1)-th cells, respectively, at the boundary. The apoplastic fluxes can then be replaced by their definitions (given by equations 8.73, 8.76 and 8.79) to introduce concentration gradients, yielding, for i = 1,2,...,m:

$$(-Ai^{j}\ B\blacksquare_{i}^{j}\ \partial ci_{i}^{j}/\partial z + Ai^{j}\ ci_{i}^{j}\ v\blacksquare^{j})|_{in} =$$

$$(-Ai^{j+1}\ B\blacksquare_{i}^{j+1}\ \partial ci_{i}^{j+1}/\partial z + Ai^{j+1}\ ci_{i}^{j+1}\ v\blacksquare^{j+1})|_{in}$$

$$[8.124]$$

If we now assume that, at the interface[20], the interstitium concentrations on both sides are the same, i.e. $ci_{i}^{j} = ci_{i}^{j+1}$ for all species and, accordingly, the densities of the interstitial solutions are equal, equation 8.124 can be broken off into two relations describing the conditions applying at any interface between 2 cells, i.e. for *convective* transfer:

---

[19] The interface can be seen as an infinitesimal impermeable buffer zone connecting the interstitium of 2 neighboring cells.

[20] The reader should realize that the present discussion concerns the description of the behavior of the system as we approach the interface. The term "at the interface" should therefore be interpreted as: "at an infinitesimal distance from the interface".

$$(Ai^{j}\, v\blacksquare^{j})|_{in} = (Ai^{j+1}\, v\blacksquare^{j+1})|_{in} \qquad [8.125]$$

and, for *diffusive* transfer:

$$(Ai^{j}\, \mathbf{B}\blacksquare_{i}^{j}\, \partial ci_{i}^{j}/\partial z)|_{in} = (Ai^{j+1}\, \mathbf{B}\blacksquare_{i}^{j+1}\, \partial ci_{i}^{j+1}/\partial z)|_{in}$$
$$i = 1,2,\ldots,m \qquad [8.126]$$

where, as can be seen, equation 8.125 will be used to link the interstitium volume average velocities at the interface and equation 8.126, the concentration variations. Note that we have not attempted here to model hydrodynamic properties of the flows occurring in the interstitium.

The boundary conditions related to the tissue center (defined in point 2) are special cases of equations 8.125 and 8.126. Restricting the dis[illegible]ssion to the infinite slab geometry, one can observe that, due to the symmetry at the center, $Ai^{j} = Ai^{j+1}$ and $\mathbf{B}\blacksquare_{i}^{j} = \mathbf{B}\blacksquare_{i}^{j+1}$, whereas the concentration gradients and the volume average velocities, being equal but of opposite signs near the interface, become, at the limit, null, i.e. $(\partial ci_{i}/\partial z)|_{o} = 0$ and $v\blacksquare|_{o} = 0$, which implies that at any time $(\partial v\blacksquare/\partial z)|_{o} = 0$.

8.3 Summary

The initial and boundary conditions required to solve the system of equations pertaining to the proposed model having been described, one has now the complete *mathematical picture* of the phenomena to be simulated. Table 8.1 summarizes the analysis of the preceding sections by presenting in a succinct manner the list of the model equations. For the sake of briefness, only the leading relations are listed. The reader should refer to the appropriate sections for the details of the accessorial equations. Complete details on the technicalities leading to the solution of this system can be found in Appendix 4. Appendix 5 contains, for its part, the listing of the computer program written to implement the numerical considerations described in the former appendix.

We will now turn our attention to presenting the results of *simulations* that were performed to validate the predictive properties of the proposed model. The first part of Chapter 9 is devoted to the description of mass transfer in the presence of an *impermeable* diffusible solute, along with a detailed analysis of the influence of some of the model parameters on such a process, whereas the second part deals briefly with transport of a *permeable* species.

Table 8.1 Summary of the model equations.

Description	Section number[1]	Equation number[2]
Mensural properties:		
- average unit cell	8.1.1	
- ECUC	8.1.2	
Transmembrane exchanges:	8.2.1(15.1.1)	
- thermodynamic forces		
transmembrane transport		8.49(A4.14-15)
symplastic transport		8.50(A4.22-23)
- flux equations		
transmembrane transport		8.59(A4.13)
symplastic transport		8.60(A4.21)
Interstitium equations of continuity:	8.2.2(15.1.2)	
- for i-th species		8.78(A4.34)
- total		8.72(A4.37)
Intracellular equations of change:	8.2.3(15.1.3)	
- volume variation		8.89(A4.44)
- concentration variation (for i-th species)		8.90(A4.43)
Supplementary equations of change:	8.2.4(15.1.4)	
- geometrical relations (ECUC mensurations)		8.92-95(A4.50-53), 8.100-101(A4.54-56)
- changes during dehydration		
ECUC volumes		8.102-103(A4.57-58)
ECUC transfer areas		8.104-105(A4.59-60)
- overall mass variation (tissue)		
i-th species		8.107(A4.68)
total		8.112(A4.67)
Initial and boundary conditions	8.2.5(15.1.5)	

---

[1] The number appearing in parentheses refers to the section in Appendix 4 which details the development of the reduced or modified form of the equations cited.

[2] The number appearing in parentheses refers to the reduced (dimensionless) or modified form of the equation (see Appendix 4 for details).

9. TISSUE BEHAVIOR: RESULTS OF SIMULATIONS AND DISCUSSION

9.1 Mass transfer in the presence of an impermeable diffusible solute

9.1.1 Test studies

The preliminary phase of the work consisted of carrying out simulations designed to test the appropriateness of the model by comparing *predicted data* to *experimental observations* taken from the literature. Two typical studies were considered, each describing the behavior of pieces of plant storage tissues immersed in osmotica containing an *impermeable* solute.

Red beet tissue

Figure 9.1 depicts the weight changes of *red beet* disks transferred from pure water to a 10 atmosphere sucrose solution (0.4088M, see discussion below). The experimental data were reported by Kohn and Dainty (1966) in their work on the measurement of the hydraulic conductivity (*Lp*, see section 2.2.4) and of the diffusional permeability in disks of red beet (*Beta vulgaris*). Each experimental point shows the mean and its standard deviation computed from the results of measurements performed on eight disks. The predicted curve was generated using a program (see Appendix 5) implemented to solve the model equations for a *hypothetical disk* subjected to the same osmotic stress, following the theoretical considerations described

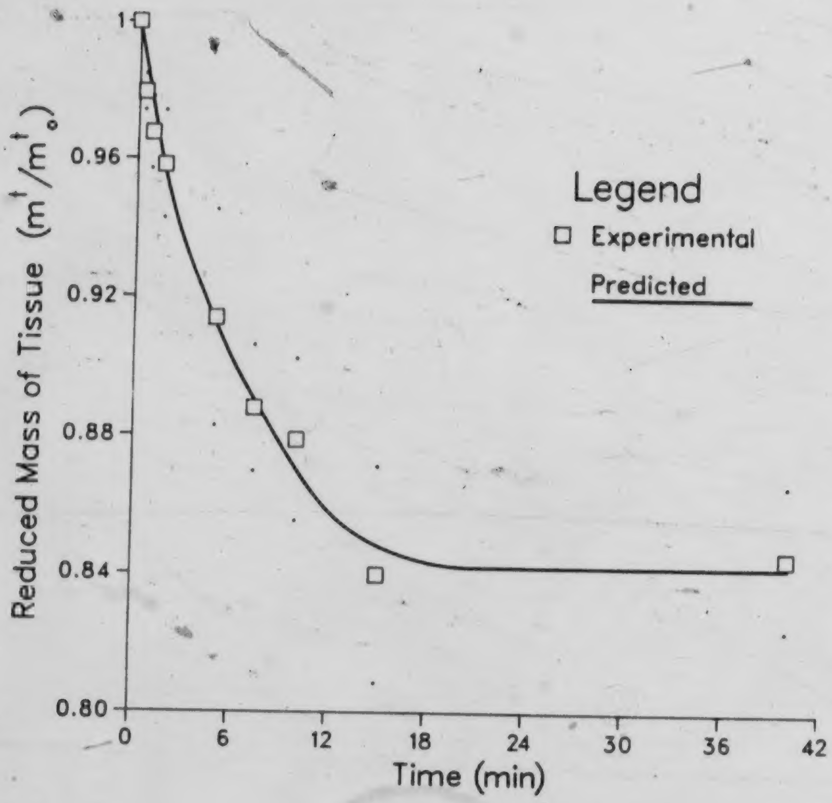

Figure 9.1 Weight changes of red beet disks transferred from pure water to a 10 atmosphere sucrose solution.

previously.

The values of the parameters utilized in this study, which are summarized in Table 9.1, were obtained partly from the above reference but mostly from other sources. This information can be divided into two types: firstly, *constants* such as molecular weights, partial molar volumes or diffusivities, but also some well documented properties and characteristics of the tissue under consideration, such as the average diameter of the cells, the initial intra- and extracellular concentrations or the initial void fraction of the tissue; and, secondly, *adjustable parameters* which by nature are known with less certainty, such as the membrane permeability coefficients, the diffusibility of the apoplast or the cell wall elastic modulus; or are simply unknown, such as the apparent cellular nonosmotic volume or the critical cellular volume.

1. *Constant* parameters. To begin, it was assumed that the solutions involved in the system, i.e. the intra- and extracellular solutions as well as the osmoticum, could be assimilated to *binary sucrose solutions* and, accordingly, could be described, using the Van't Hoff law, by *effective* or *apparent concentrations* (equation 4.7), which would yield, through the following relation, the *osmotic pressures* quoted in the references. It is indeed usual practice in animal and plant physiology literature to quote concentrations in *pressure* units, i.e. in atmospheres or bars. This approach has the main advantage of describing the

Table 9.1 Summary of parameter values used in red beet test study.

Description	Units	Parameter value
Osmoticum characteristics:		
- temperature	°C	25
- partial molar volumes	m^3 $kgmol^{-1}$	
water		18.016×10^{-3}
sucrose		211.0×10^{-3}
- molecular weights	kg $kgmol^{-1}$	
water		18.0
sucrose		342.3
- diffusivity of sucrose in water		
$D_{\blacksquare,\infty}$	m^2 s^{-1}	5.23×10^{-10}
a_0	dimensionless	3.410×10^{-3}
a_1	dimensionless	-0.718
- sucrose concentration	kgmol m^{-3}	0.4088
Initial tissue characteristics:		
- corrected thickness	m	3.6×10^{-4}
- disk diameter	m	7.5×10^{-3}
- tissue volume fractions	dimensionless	
ϕw		0.05
ϕs		0.05
- mass of disk	kg	1.65×10^{-5}
- interstitium sucrose concentration	kgmol m^{-3}	0
Initial cell characteristics:		
- cell diameter	m	6.117×10^{-5}
- nonosmotic volume	dimensionless	0.27
- critical volume	dimensionless	0.77
- cell wall diffusibility	dimensionless	0.3
- αp	dimensionless	0.01
- elastic modulus (constant)	N m^{-2}	3.3×10^6
- membrane permeabilities	kgmol N^{-1} s^{-1}	
Þm(water)		1.14×10^{-12}
Þp(water)		1.14×10^{-10}
- equivalent sucrose concentration	kgmol m^{-3}	0.2863

The reader is referred to the text for details on the data sources.

molecular potential of solutions of unknown composition through one colligative property, the *osmotic pressure* (see section 4.1.1), that is:

$$\pi = \Re T\, c(\mathrm{eff}) \qquad [9.1]$$

where π is the osmotic pressure of the binary solution which would *ideally* be induced by adding c moles per liter of solute in water. T is the temperature of the studied system, which was assumed to be 298.15°K (25°C) due to the unavailability of this information in the above reference.

It is important to recall that the model has been designed assuming that the solutions involved in the system are *ideal* and *dilute*, such that the relations described in section 8.2.1 can be used. As discussed in section 4.1.1, it is through the concepts of the *apparent concentration* and the *apparent cellular nonosmotic volume* that we intend to *correct* for this obvious discrepancy from reality. Furthermore, we will suppose that, in the case of studies involving *impermeable* solutes such as sucrose or mannitol (see below), the only species which crosses the membranes is *water*. This assumption can be justified in view of the fact that the other permeable species present initially in the tissue (ionic species mostly) are far less concentrated than water and, consequently, can be neglected in the context of a macroscopic description.

Hence, referring to Kohn and Dainty (1966), the osmoticum concentration (assumed to stay constant throughout the experiment) corresponds to an apparent sucrose concentration of 0.4088 kgmol m^{-3} (0.4088 M). The extracellular solution, for its part, was taken as being *pure water* since the disks used in the experiment, after being washed in running tap water, were transferred to aerated distilled water in which they were stored for at least 48 hours. This is further supported by Ferrier and Dainty (1977) and Green, Ferrier and Dainty (1979), who state that such pretreatment not only ensures that the tissue is initially at *full turgor*, but also that the intercellular spaces and cell wall volumes are likely to be water filled since, *in vivo*, solute concentration in the apoplast is already quite low. The intracellular concentration at full turgor was taken as 0.2863 kgmol m^{-3} (0.2863 M), which corresponds to a value lying half way between osmotic pressures of 6 to 9 bars quoted by Ferrier and Dainty (1978) for red beet cells. The latter value is equivalent, as discussed previously, to assuming that the intracellular solution is composed of a *fictitious impermeable solute* inducing, at full turgor, an osmotic pressure of 7 bars. This solution can be assimilated to an *equivalent sucrose solution* since the Van't Hoff law (relation 9.1) is independent of the nature of the solute.

Partial molar volumes and molecular weights were obtained from standard tables (Weast, 1977; Moelwyn-Hughes, 1961) and are, respectively for water and sucrose: 18.016×10^{-3} and 211.0×10^{-3} m^3 $kgmol^{-1}$ for the partial molar volumes and 18.0 and 342.3 kg $kgmol^{-1}$ for the molecular weights. The description of the concentration dependence of the diffusion coefficient of sucrose in water was approached by fitting a *polynomial* of the following form to measurements obtained at different concentrations (Landolt-Börnstein tables, 1969):

$$\ln (D\blacksquare_i/D\blacksquare_{i\infty}) = \Sigma_k a_k c_i^k \; ; \; k = 0,1,\ldots,n \qquad [9.2]$$

where $D\blacksquare_i$ is the diffusivity of species i at a concentration c_i in *water*, $D\blacksquare_{i\infty}$ is the diffusivity at *infinite dilution* and a_k is the k-th coefficient of the polynomial of degree n. For sucrose in water, a polynomial of degree 1, i.e. a straight-line relationship, was found adequate ($R^2=0.9973$) to describe the above dependence, leading, for a value of $D\blacksquare_{i\infty}$ at 25°C of 5.23×10^{-10} m^2 s^{-1}, to coefficients of 3.410×10^{-3} and -0.718 for a_0 and a_1 respectively (for concentrations ranging from 0 to 2.6 M at 25°C). Following Kohn and Dainty's arguments it was assumed that resistance to mass transfer at the surface of the hypothetical disk could be neglected since the thickness of the unstirred layer, estimated by the authors to be of the order of 100 µm, would have little effect on the transport phenomena as compared to the resistances encountered in the tissue.

In terms of tissue characteristics, the disks used to carry out the experiment were estimated to be about 4.2×10^{-4} m thick[21]. The authors, however, did not quote explicitly the diameter of the pieces. They simply reported that: "it was always possible to select tissue from between the same *two rings* of vascular tissue and to ensure that a large proportion of the cells in a given disk were parenchymatous". It was therefore assumed that a diameter of 7.5 x 10^{-3} m would represent a reasonable guess (at least an upper limit). The geometry of the hypothetical disk was accordingly assimilated to an *infinite slab* for the calculations. To take into account the *cut cells* on the exposed surfaces of the disks, a fraction of 15% of the total volume of the piece was considered as damaged cells, leading to a *corrected* thickness of 3.6 x 10^{-4} m (see section 8.2.4). This estimate corresponds to the lower limit of a range of 15 to 20% reported by Pitman (1963) and to the value quoted by Cram (1968) for carrot tissue of similar dimensions. The average cell diameter was obtained from a size-distribution of cell radii in beet root performed by Myers (1951), which yields an average value of 6.117 x 10^{-5} m (61.17 μm). The initial cell wall and intercellular free space volume fractions were both taken as occupying 5% of the total volume, as discussed by Pitman (1963). The initial mass of the piece was evaluated from the *uncorrected* volume, i.e. 1.57 x 10^{-8} m^3, assuming a bulk density of 1050 kg m^{-3} for beet root tissue (value lying halfway between bulk densities of 995.5 kg m^{-3} and 1111 kg m^{-3} reported by Glinka

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[21] The thickness was evaluated from the value of the half-time of the experimental weight change, i.e. the time

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[21] (cont'd) required to reach half of the final total weight loss (≅4 min), in conjunction with a plot showing the half-time values as a function of the mean disk thickness squared (Figure 2 in Kohn and Dainty [1966]).

and Reinhold [1964, 1971] for carrot tissue), leading to an initial mass of 1.65×10^{-5} kg.

2. *Adjustable* parameters. As discussed previously, the adjustable parameters consist mostly of properties of the tissue which are difficult to assess experimentally, rendering these estimates, if available, less reliable. They therefore leave some room for adjustment in an attempt to improve the agreement between predicted and observed data. Since the role of these parameters in the general behavior of the studied system will be examined in detail in section 9.1.2, we will restrict the discussion to presenting the "optimized" values used to generate the predicted curve. It should be pointed out that they do not correspond to *least-squares estimates* since the optimization procedure was performed on a *trial-and-error* basis by adjusting the parameter values until a satisfactory fit was obtained.

In this manner, the *water* macroscopic permeability coefficient of the plasmalemma, Þm_{ii} (for $i \equiv$ water, see section 8.2.1) for the system under consideration was adjusted to a value of 1.14×10^{-12} kgmol N^{-1} s^{-1}, which can be compared to initial estimates of 2.33 and 1.94×10^{-12} kgmol N^{-1} s^{-1} quoted respectively by Ferrier and Dainty (1978) and Myers (1951) for red beet cells[22]. The permeability of the plasmodesma pseudo-membrane, Þp_{ii}, was taken as 1.14×10^{-10} kgmol N^{-1} s^{-1}. This estimate corresponds to an increase in permeability of the latter membrane (as compared to the plasmalemma permeability) of 100-fold, following the conclusions reached by Tyree (1970), who showed that the permeability of the plasmodesmata to water was at least *two orders* of magnitude higher than the permeability of the plasmalemma to that species (see also Anderson [1976]). The symplasm pathway was taken as occupying 1% of the plasmalemma area as reported by Ferrier and Dainty (1977). The cell wall elastic modulus was assumed constant and adjusted to 3.3 x 10^6 N m^{-2}, which is almost identical to an estimate of 3.7 x 10^6 N m^{-2} reported by Ferrier and Dainty (1978) for red beet tissue. The diffusibility of the cell wall complex, ψw, was computed from equation 8.7 using a value of 0.6 for the cell wall void fraction, ϵw (Ferrier and Dainty, 1977) and assuming a cell wall tortuosity, τw, of 2, leading to an estimate for ψw of 0.3. This value should represent at least an upper limit since the tortuosity of the cellulosic matrix could be more important (see Nobel, 1983).

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[22] Note that these permeabilities have been transformed to comply with the unit system used in this work (see section 5.2.1).

The apparent cellular nonosmotic volume, which controls, as we will see, the equilibrium state of the system, led to a reasonable fit when its value was set (in reduced form, $\underline{V}d$) to 0.27, which can be compared qualitatively to estimates of 0.24 reported for yeast cells (Levin *et al.*, 1979) or of 0.42 found earlier for human granulocytes (see section 4.4). The lower values appear to substantiate the presence in the former units of an important vacuolar volume, occupying up to 70% of the cellular volume (Pitman, 1963), where up to 98% of the cell water may be contained (Slayter, 1967). The critical cellular volume (see section 8.1.1), the role of which will be described in section 9.1.2, was set, for its part, to 0.77 (in reduced form), which lies below the equilibrium relative volume reached by the cells (≅0.78).

It remains to discuss the values ascribed to the adjustable *operational* parameters controlling the numerical techniques employed to solve the model equations (see sections 15.2 and 15.3). Our main concern was to select values that would, on one hand, minimize computational costs and, on the other hand, ensure sufficient stability and convergence on the true solution. As for the preceding parameters, this adjustment was performed on a trial-and-error basis by observing the behavior of the solute concentration profile in the interstitium at the *onset* of the simulation. It was found that the *initial time increment* controlling the first iteration of the Crank-Nicolson algorithm and, as discussed in section 15.3, specifying the initial time interval of integration of the intracellular and geometrical equations of change, had to be relatively small, i.e. in the order of 0.1 second, to ensure proper establishment of the concentration profile. This requirement is a direct outcome of the nature of the ordinary differential equations to be solved, which, due to their dependence on the thermodynamic forces present in the system (which assume their maximum values at the early stage of the dehydration process), are particularly *stiff* (see section 15.3). The introduction of an acceleration procedure with an acceleration factor of 1.1 (see equation A4.127) led to stable development of the solution while minimizing computational time. The acceleration procedure was stopped when the time step reached 30 seconds to allow appropriate printing intervals. It was found, however, that this limiting value could not exceed 60 seconds due to noticeable fluctuations of the solution. A convergence criterion of 0.01 was selected to govern the iterative procedure pertaining to the Crank-Nicolson algorithm (see section 15.2.1), whereas a relative error bound of 10^{-5} (see subroutine DGEAR, User's manual, IMSL Library, 1980) controlled the predictor-corrector method used to integrate the ordinary differential equations cited above. The reader is referred to Appendix 5, subroutine CELLCH, for more details on the implementation of subroutine DGEAR.

Potato tissue

For the second test study, the results of which are presented in Figure 9.2, the behavior of *potato* disks (*Solanum tuberosum*), transferred from pure water to a 13 atmosphere mannitol solution (0.5350M), was simulated and compared to experimental weight changes reported by Stuart (1973). The model parameters, summarized in Table 9.2, were, as in the previous study, obtained from various sources and are as follows:

Table 9.2 Summary of parameter values used in potato test study.

Description	Units	Parameter value
Osmoticum characteristics:		
- temperature	°C	23
- partial molar volumes	$m^3\ kgmol^{-1}$	
water		18.016×10^{-3}
mannitol		119.0×10^{-3}
- molecular weights	$kg\ kgmol^{-1}$	
water		18.0
mannitol		182.17
- diffusivity of mannitol in water		
$D_{\blacksquare i\infty}$	$m^2\ s^{-1}$	6.665×10^{-10}
a_0	dimensionless	1.509×10^{-5}
a_1	dimensionless	-0.317
- mannitol concentration	$kgmol\ m^{-3}$	0.5350
Initial tissue characteristics:		
- corrected thickness	m	0.8×10^{-3}
- disk diameter	m	1.4×10^{-2}
- tissue volume fractions	dimensionless	
ϕw		0.05
ϕs		0.03
- mass of disk	kg	1.67×10^{-4}
- interstitium mannitol concentration	$kgmol\ m^{-3}$	0
Initial cell characteristics:		
- cell diameter	m	1.80×10^{-4}
- nonosmotic volume	dimensionless	0.035
- critical volume	dimensionless	0.80
- cell wall diffusibility	dimensionless	0.3
- αp	dimensionless	0.01
- elastic modulus		
β_1	dimensionless	3.0
β_2	$N\ m^{-2}$	1.5×10^6
- membrane permeabilities	$kgmol\ N^{-1}\ s^{-1}$	
Þm(water)		7.00×10^{-12}
Þp(water)		1.02×10^{-9}
- equivalent mannitol concentration	$kgmol\ m^{-3}$	0.1

The reader is referred to the text for details on the data sources.

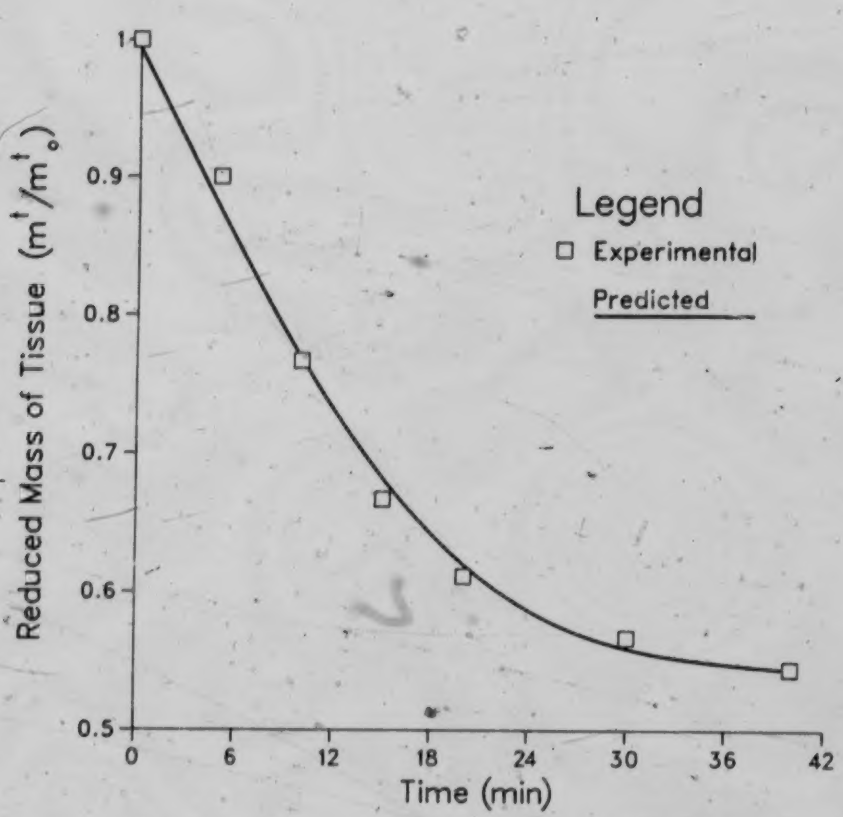

Figure 9.2 Weight changes of potato disks transferred from pure water to a 13 atmosphere mannitol solution.

1. *Constant* parameters. Contrary to the analysis of red beet tissue, the solutions composing the potato system were assimilated to equivalent *mannitol* solutions, since this was the impermeable solute used by Stuart in his analysis. Accordingly, referring to his paper, apparent concentrations of 0.5350 kgmol m^{-3} (0.5350 M) and 0.1 kgmol m^{-3} (0.1 M) were computed (for a system at 296.15°K [23°C]) for the osmoticum and initial intracellular concentrations, respectively. The latter value was derived from an estimate of 2.4 atmospheres obtained from measurements performed on fresh potato tubers, as reported by Salisbury and Ross (1969). The extracellular solution was taken as pure water since the potato disks used in Stuart's experiment underwent a pretreatment similar to the one utilized by Kohn and Dainty for their red beet samples.

Partial molar volumes and molecular weights were obtained again from Weast (1977) and Cram (1984) and are, respectively, for water and mannitol: 18.016×10^{-3} and 119.0×10^{-3} m^3 $kgmol^{-1}$ for the partial molar volumes, and 18.0 and 182.17 kg $kgmol^{-1}$ for the molecular weights. The composition dependence of the diffusivity of mannitol in water, for concentrations ranging from 0 to 0.8 M, was found to be well approximated by a straight-line relationship with coefficients a_0 and a_1 (see relation 9.2) of 1.509×10^{-5} and -0.317, respectively (R^2=0.9998), with a diffusivity at infinite dilution at 25°C of 6.665×10^{-10} m^2 s^{-1} (Landolt-Börnstein tables, 1969).

As reported by Stuart (1973), disks of 1.4 x 10^{-2} m in diameter and 1.0 x 10^{-3} m thick were used to carry out the experiment. A corrected thickness of 0.8 x 10^{-3} m was used in the calculations to take into account layers of about 0.1 x 10^{-3} m thick of dead cells formed on each surface on slicing (Poole, 1976). Infinite slab geometry was chosen as representative of the system under consideration and it was assumed that the resistance to mass transfer on each surface could be neglected, as discussed previously. The average cell diameter was obtained from diametric measurements performed by Chung (1979), leading to an average value of 1.80 x 10^{-4} m (180 µm). The initial intercellular free space and cell wall volume fractions were taken as representing, respectively, 3% (Sterling, 1966; Crapiste and Rotstein, 1982) and 5% of the total tissue volume. The latter fraction is identical to the value used for red beet and agrees with microscopic observations which indicate that the cell wall and intercellular volumes of many tissues constitute 7 to 10% of the total tissue volume (Salisbury and Ross, 1969).

The initial mass of the hypothetical piece was taken as 1.67 x 10^{-4} kg, which corresponds to 1/6-th of the weight of the "6-disks" samples (≅ 10^{-3} kg fresh weight) used by Stuart in his experiment. This estimate is in agreement with an average bulk density of 1070 kg m^{-3} for potato tuber quoted by Davis (1962).

2. *Adjustable* parameters. The water macroscopic permeabilities for the plasmalemma, Þm_{11}, and the

plasmodesma pseudo-membrane, $\text{Þ}p_{II}$, were adjusted, respectively, to 7.00×10^{-12} and 1.02×10^{-9} kgmol N^{-1} s^{-1}, which represents a 7-fold increase in permeability for plasmalemmal transport as compared to beet cells, and a ratio of plasmodesma to plasmalemma permeability of 145:1 for the present system. The former optimum is relatively close to an initial estimate of 5.85×10^{-12} kgmol N^{-1} s^{-1} proposed by Heinrich (1962) for plasmalemma of potato cells (Rotstein and Cornish, 1978b). As for the permeability of the plasmodesma complex, the large ratio is in accordance with the findings of Tyree (1970), which were outlined in the previous section.

Let us note that, in terms of the results of the simulations for red beet and potato tissues, similar fits could have probably been obtained by keeping the ratio of plasmodesma to plasmalemma permeability to a lower value and by increasing the fraction of total cell membrane surface area occupied by plasmodesmata beyond 1%. However, this would have been contrary to the conclusions of Robards (1976), who notes that this fraction, most likely, does not exceed that limit in higher plants.

The cell wall elastic modulus, ξ, contrary to the previous simulation, was made *pressure dependent* since the coefficients appearing in equation 8.42 were available. Hence, referring to Nilsson *et al*. (1958), the variation of ξ with respect to the intracellular hydrostatic pressure, β_1, and the value of ξ at incipient plasmolysis, β_2, were taken, respectively, as 3.0 (dimensionless) and 1.5 x 10^6 N m^{-2}. The diffusibility of the cell wall complex was supposed similar to the value computed for red beet tissue, i.e. ψw = 0.3, which seems reasonable since the cellulosic microstructure should be relatively similar in both systems.

The most important divergences between red beet and potato tissue characteristics, besides their membrane permeabilities, appear in fact when one examines the "optimized" reduced nonosmotic volume and, to a lesser extent, the reduced critical cellular volume, required to achieve the fit presented in Figure 9.2. Values of 0.035 and 0.80, respectively, were necessary to describe the behavior of the present system comparatively to values of 0.27 and 0.77 used in the previous analysis. Although any attempt to explain these differences is hazardous, especially in view of the nature of these parameters, the lower magnitude of the nonosmotic volume in potato cells might be partly related to the presence in these units of a large amount of starch granules (Chung, 1979) which may act through their sorptional properties as "*water pools*", thus increasing the apparent "solution" volume of the system. This influence of starch, in starch-containing foodstuffs, has been examined recently by Crapiste and Rotstein (1982). The authors showed that this component was indeed playing a major role as controlling factor in osmotic exchanges, which would tend to support the above hypothesis. As for the critical cellular volume, the "optimized" value lies well above the equilibrium relative volume reached by the cells (≅ 0.22). Finally, we should note that the operational parameters employed in the red beet simulation were found perfectly adequate for the present study and were therefore not altered.

Conclusions with respect to the performance of the proposed model

The results obtained by simulating the behavior of hypothetical disks based on the preceding parameter values are quite encouraging, as can be seen in Figures 9.1 and 9.2. Although the "forms" of the fitted curves are relatively simple, one must remember that they correspond to *macroscopic* representations of complex *microscopic* phenomena which are described by the model through parameters having a *physical* meaning, i.e. parameters which are related to microscopic properties of the systems considered. This is in contrast to similar results that could have been obtained by fitting simpler models based on purely *empirical* parameters. This distinction is crucial in the present discussion as it confirms that in its present form the proposed model can be used not only to simulate the behavior of real structures but also to analyse the influence of tissue characteristics on the response of the cellular matrix.

As to the numbers of adjustments and assumptions required to achieve the fits, one must realize that, in the context of an optimization process, the observed good agreement between experimental and predicted data is attainable for a model based on physical parameters only if it is a sufficient representation of the reality. If the simulations are carried out with parameter values which agree with the physical reality that they represent, one can be confident of the potential of the tested model.

9.1.2 Parametric studies

The role of specific parameters on the overall tissue behavior can be illustrated quite effectively if one takes advantage of the predictive characteristics of the model to generate the response of the studied system to variation in the magnitude of these parameters. The process consists essentially in selecting a *reference behavior* (which determines the "*base*" parameter values to be used) and to alter the property of interest, within a certain range of possible values, keeping all other parameters constant.

Four major properties, most of them presented earlier as "*adjustable*" parameters, will be examined in the following analyses, namely: the membrane permeabilities, the diffusibility of the extracellular continuum, the relative cellular nonosmotic volume and the critical cellular volume. For that purpose, the "*optimized*" curves presented in Figures 9.1 and 9.2 will play the role of the reference lines and will hereafter be referred to as *reference* or *test curves*.

Membrane permeability coefficients

Figures 9.3 and 9.4 illustrate the effect of varying the *plasmalemma* permeability on the course of osmotic exchanges in red beet and in potato tissue, respectively. To carry out these simulations, the magnitude of the parameter for each system was increased then decreased by a factor of 2.5 from its reference value, while keeping the ratio plasmodesma to plasmalemma permeability constant.

As anticipated, an increase or decrease in the permeability of the cell membrane modifies drastically the rate of dehydration of both tissues. For instance, referring to Figure 9.3 (red beet case), one can observe that the total time for reaching equilibrium varies from approximately 15 minutes when the permeability is high (2.86 x 10^{-12} kgmol N^{-1} s^{-1}) to 25 minutes when it is reduced to its lowest value (0.46 x 10^{-12} kgmol N^{-1} s^{-1}). The same trend holds for the *half-time* for equilibration, i.e. the time required to achieve half the total weight loss, with estimates of 2.8 and 5.9 minutes, respectively. In the case of potato tissue (Figure 9.4), times of 24 minutes, for a maximum permeability of 1.75 x 10^{-11} kgmol N^{-1} s^{-1}, and greater than 40 minutes, for the lower limit (2.80 x 10^{-12} kgmol N^{-1} s^{-1}), are found for the total time for reaching equilibrium, whereas times of 5.5 and 17.9 minutes, respectively, are necessary to achieve half the total weight loss.

These changes in the rate of dehydration of tissues have been reported by numerous authors (Glinka and Reinhold,

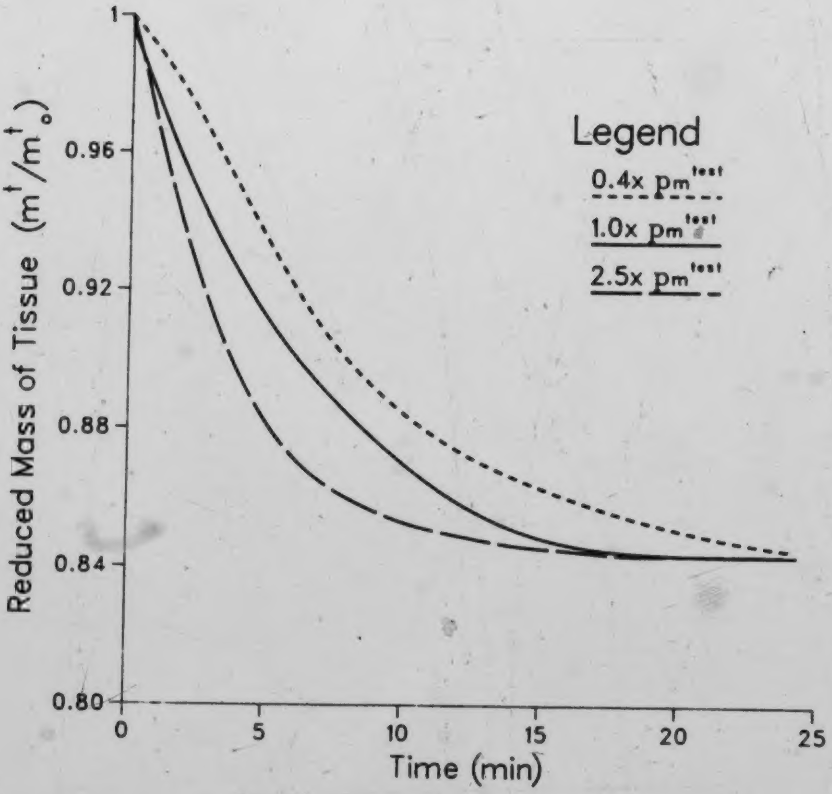

Figure 9.3 Effect of varying the plasmalemma permeability on the course of osmotic exchanges in red beet tissue.

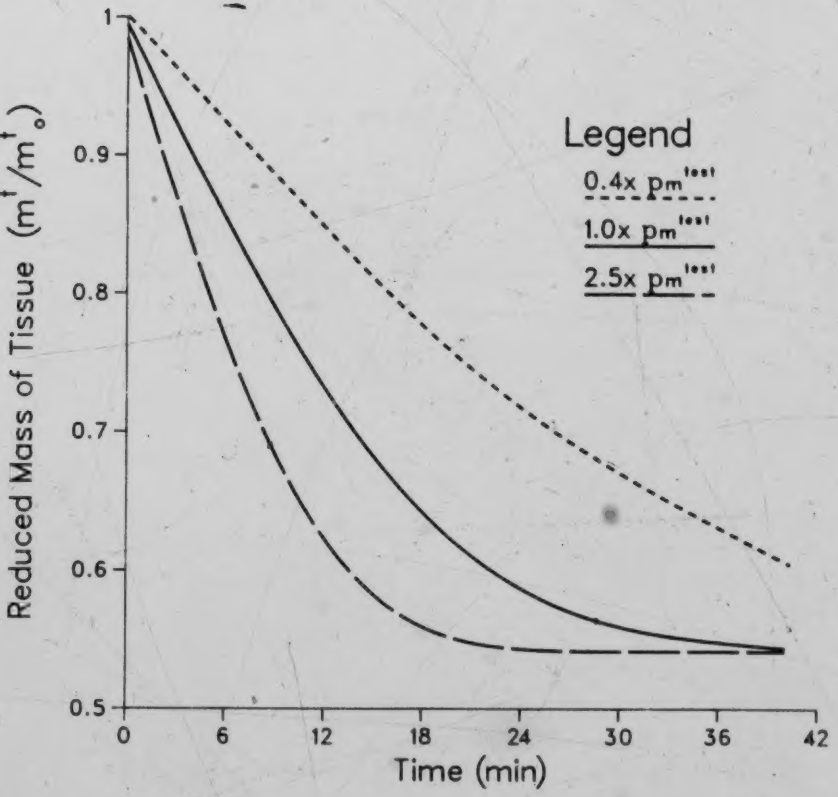

Figure 9.4 Effect of varying the plasmalemma permeability on the course of osmotic exchanges in potato tissue.

1964, 1971 and 1972; Virgin, 1955; Stuart, 1973) who were interested in studying the influence of various chemical substances, mostly auxins or antiauxins, on the permeability of the cellular membrane. They showed that treatments with such substances could shift the reference curves, i.e. the "*untreated*" lines, in a similar fashion, either upward (decreased permeability) or downward (increased permeability), depending on their effects on the selective properties of the membrane. In all cases, though, the equilibrium weights would converge on the same final values, as predicted by the model, unless the cells were killed during the treatment, in which case the tissues would lose more weight since part of the intracellular material would seep through the membrane and be washed out (Glinka and Reinhold, 1972).

The observed convergence is to be expected if we assume that the cells retain their osmotic behavior during the treatment. As a close inspection of the model equations reveals (it should be emphasized that the model considers the cells as undisrupted osmometers, i.e. that the cell integrity is not jeopardized by the osmotic process), the *asymptotic* behavior of the systems, i.e. their responses as time approaches infinity, is *independent* of the values taken by the plasmalemma permeability.

The effect on the dynamic of dehydration of varying the magnitude of the permeability of the *plasmodesma pseudomembrane* is presented for both tissues in Figures 9.5 (red beet case) and 9.6 (potato case). In these studies, all parameters were kept constant, except for the ratio plasmodesma to plasmalemma permeability, which was increased from 0:1, i.e. precluding symplastic transport, to 145:1, the optimum found for potato tissue. The ratio 38:1 was introduced to test the behavior of the systems for the value used by Molz (1976) in his study of apoplastic and symplastic transport in plant tissue. The ratio 84:1 (appearing in Figure 9.6), on the other hand, represents only a convenient point located more or less halfway between Molz's ratio and the optimum.

As can be observed, the rate of dehydration of both systems increases as permeability increases, which is in agreement with our previous observations. The influence of the plasmodesma permeability on the overall response of the tissues indicates furthermore that the role of the symplast in the behavior of these systems cannot be ignored (at least in the case of the potato tissue), especially in view that the plasmodesmata occupy barely 1% of the total cell membrane surface area.

The effect of gradually improving symplastic transport appears to result in a corresponding increased control of the pathway on the course of osmotic exchanges during the *middle* phase of the process, contrary to more global changes observed in varying the plasmalemma permeability (see Figures 9.3 and 9.4). Figures 9.5 and 9.6 reveal that the rate of weight loss in the early stage of the dehydration is

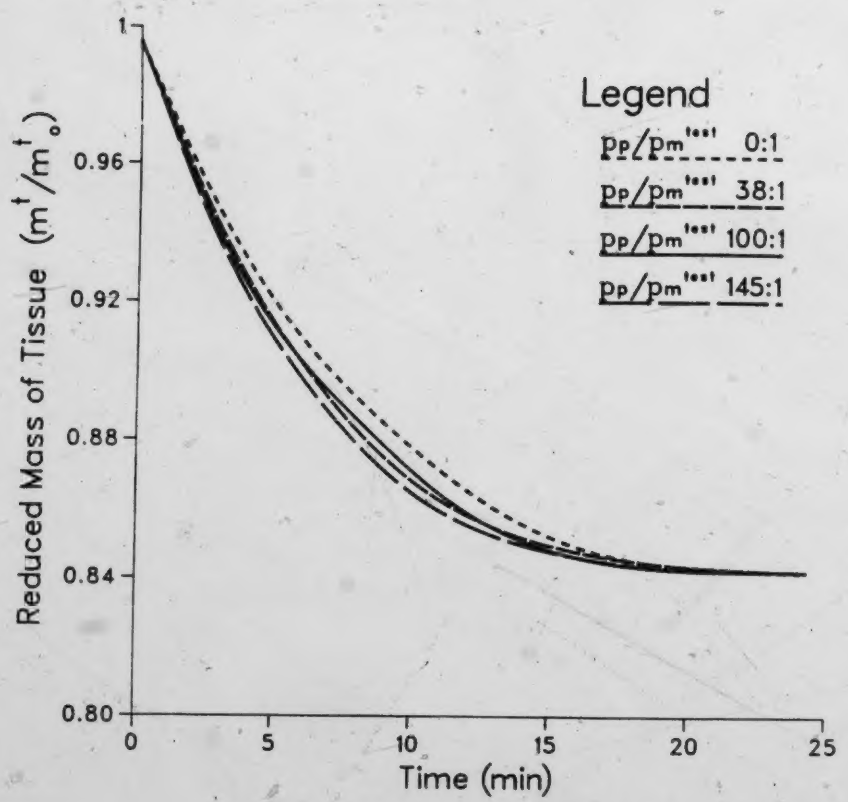

Figure 9.5 Effect of varying the permeability of the plasmodesma pseudo-membrane on the course of osmotic exchanges in red beet tissue.

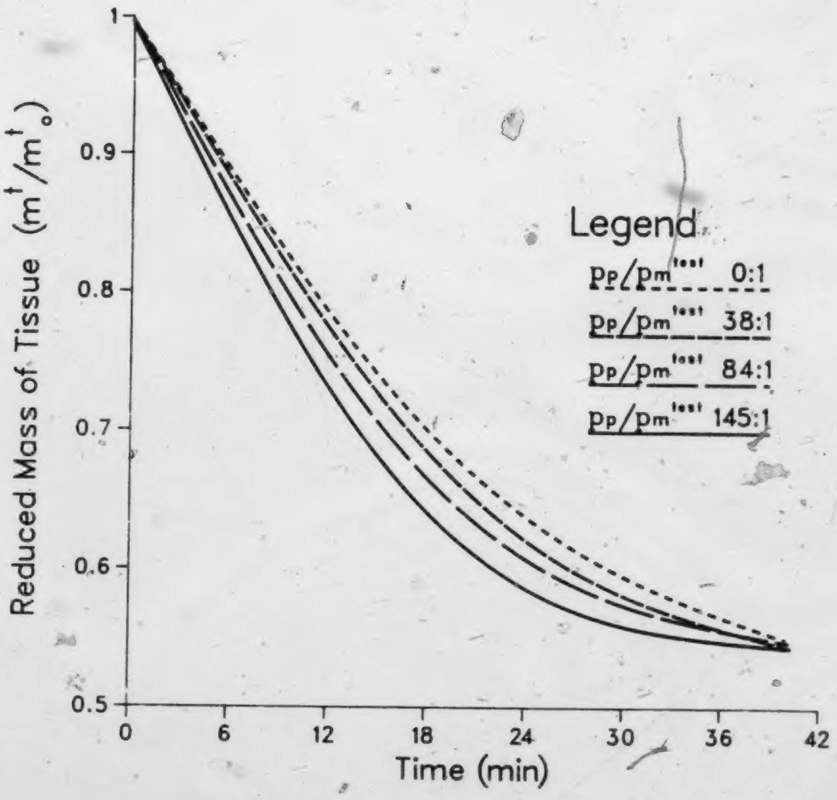

Figure 9.6 Effect of varying the permeability of the plasmodesma pseudo-membrane on the course of osmotic exchanges in potato tissue.

relatively unaffected by an increased plasmodesma permeability, whereas its dependence upon the value ascribed to that parameter becomes significant as time passes.

This phenomenon could be attributed in part to a gradual shift from transmembrane and apoplastic transport to symplastic transport (as favored pathway for water transfer in the tissue) as the apoplast becomes slowly less "efficient" due to the accumulation of the diffusing osmoticum solute in the extracellular continuum and/or due to structural reorganization of the tissue. To that respect, it is to be noted that, as the ratio plasmodesma to plasmalemma permeability approaches 100:1, the magnitude of symplastic *flows* becomes equivalent (for equal forces) to transmembrane flows since the increase in permeability cancels the effect of the small transfer area occupied by the plasmodesmata (≅1% of total membrane surface area). The symplast can in that case "take charge" of an important fraction of the transport and thus maintain the effectiveness of the dehydration process, as can be seen by the gradual shift of the curves. Note however that, although the phenomenon is noticeable in both systems, symplastic transport seems to play a substitutive role solely in *potato* tissue, whereas it appears relatively unimportant in red beet. This interesting outcome can be compared to a similar result obtained by Ferrier and Dainty (1977), showing that the main path of water flow in the red beet structure is the *apoplast* rather than the symplast. This difference in behavior could be related potentially to the relative "woodenness" of red beet tissue, which, in comparison to the much "softer" potato structure, could lead to a substantial increase in extracellular free space volume as dehydration progresses, thus improving apoplastic transport. That hypothesis will be returned to later on.

Diffusibility of extracellular continuum

In this section we will examine the influence of the *diffusibility of the extracellular continuum* on the course of osmotic dehydration of a given tissue. More specifically, we will consider the effect of varying the *cell wall* diffusibility on the process, since this parameter represents, by virtue of the approach adopted to derive equation 8.9, the sole measure of the resistance offered by the apoplast *microstructure* to diffusing molecules.

The present simulations were carried out with reference to the test studies presented earlier. Values of 0.1, 0.3 (reference), 0.6 and 1.0 were ascribed successively to the diffusibility of the cell wall, ψw, yielding the transients shown in Figures 9.7 (red beet case) and 9.8 (potato case). The first three quantities correspond (referring to equation 8.7) to assuming cell wall tortuosities, τw, of 6, 2 and 1, respectively, for a constant wall void fraction, ϵw, of 0.6, as suggested by Ferrier and Dainty (1977) for red beet tissue, while the latter value is introduced to simulate the hypothetical case of a cell wall having no hindrance effect, i.e. as if the molecules were diffusing in "solution".

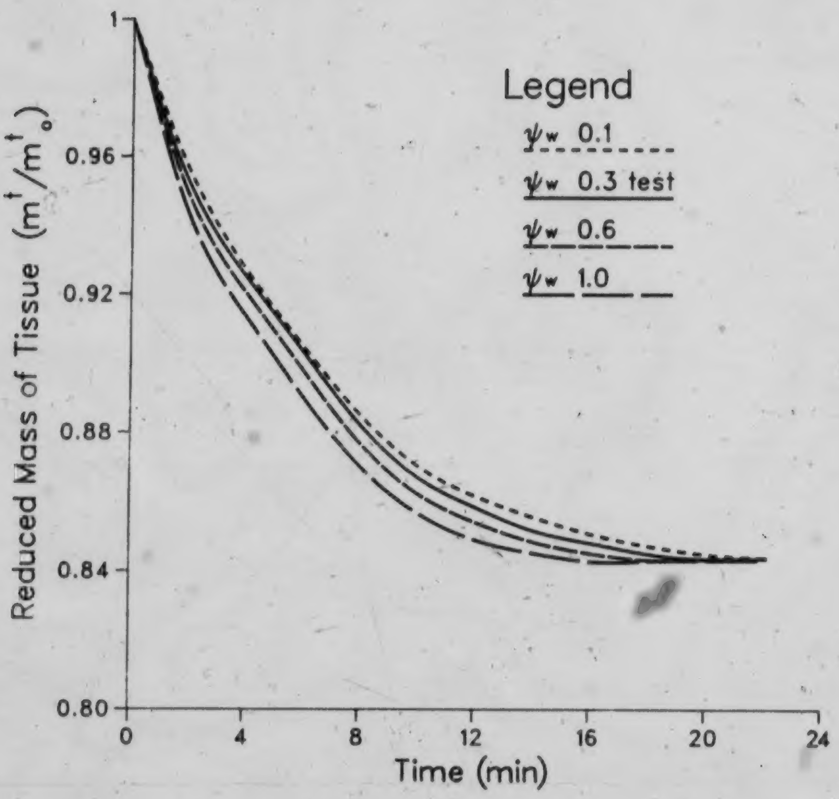

Figure 9.7 Effect of varying the diffusibility of the cell wall complex on the course of osmotic exchanges in red beet tissue.

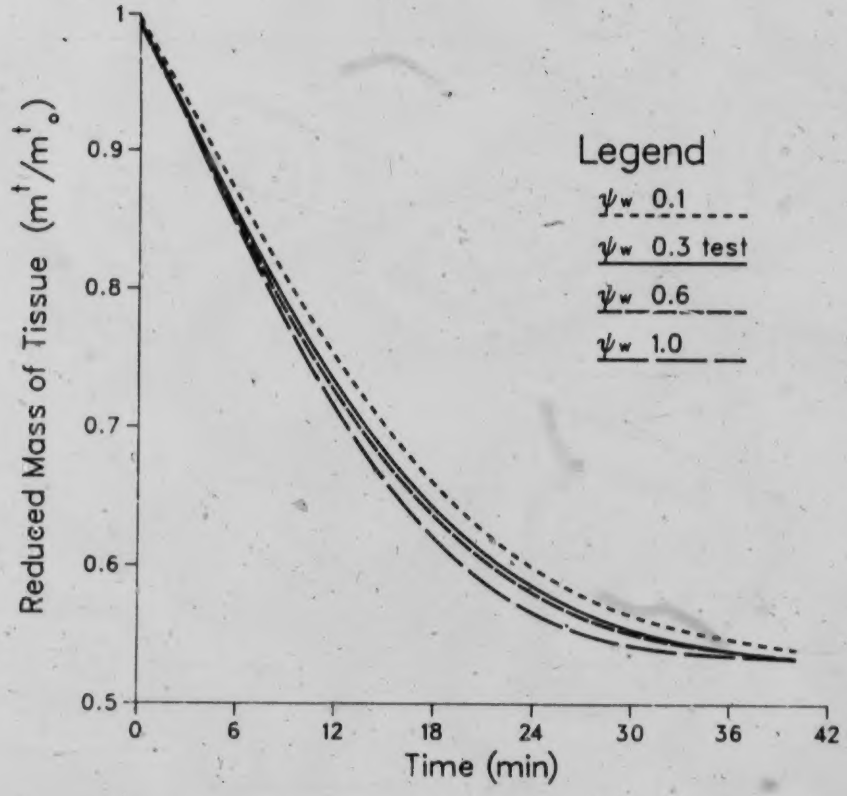

Figure 9.8 Effect of varying the diffusibility of the cell wall complex on the course of osmotic exchanges in potato tissue.

As can be seen, the course of osmotic exchanges in these systems is not altered to the extent observed previously when their plasmalemma permeabilities were modified (see Figures 9.3 and 9.4). In fact, the variations are quite similar to the ones presented when the influence of the plasmodesma permeability was analysed (see Figures 9.5 and 9.6). The difference between transport in an unhindered apoplast (ψw = 1.0) and in the most restrictive environment (ψw = 0.1) results, for example, in an increase in the half-time for equilibration of approximately 28% (3.4 to 4.4 minutes) and 20% (8.7 and 10.4 minutes), for red beet and potato tissues, respectively. This is to be compared to values of 111% (2.8 to 5.9 minutes) and 226% (5.5 to 17.9 minutes) when the plasmalemma permeabilities are increased or decreased by a factor of 2.5 from their reference values, or to increases of 15% (4 to 4.6 minutes) and 34% (9.6 to 12.9 minutes) when the plasmodesma to plasmalemma permeability ratio is varied from 0:1 to 145:1.

These results tend to show that the response of the tissues is more sensitive to changes in the cell membrane permeability than alterations in the diffusibility of the apoplast or in the permeability of the plasmodesmata. This suggests that, although the last two parameters have a definite effect on the osmotic exchanges, the *plasmalemma* represents the major resistance to mass transfer in these systems. It is important however to realize that this statement does not lessen the role in the diffusion phenomena of the apoplast microstructure or the symplast, as can be seen, for instance, by the obvious shift of the concentration profile of diffusing sucrose in red beet tissue, in response to variation of the diffusibility of the apoplast (Figure 9.9). It simply "grades" the contribution of each factor to the overall response of the tissues, as proposed by Ferrier and Dainty (1977) when describing the hierachy of resistances to water flow in red beet tissue.

Apparent cellular nonosmotic volume

The *apparent cellular nonosmotic volume*, Vd, the role of which will now be considered, is by definition (see section 4.4) a *correction* factor of purely *empirical* nature which is introduced to ensure the the cells comprising the structures under consideration obey the *Boyle-Van't Hoff* law (equation 4.46), that is, their *equilibrium* volume varies *linearly* with the inverse of the osmotic pressure of the solution surrounding them. More precisely, this factor is a measure of the *apparent "non-solution"* volume of the cells, i.e. the fraction of total cellular volume which appears not to take part in osmotic exchanges. Variations in the magnitude of this parameter determine therefore, for each individual cell, the extent of dehydration necessary to balance the extracellular thermodynamic forces induced as the osmoticum solute diffuses in the structure. In terms of tissue behavior, this should be reflected by a direct control on the total quantity of water lost by the system as it slowly equilibrates with the osmoticum, since Vd fixes

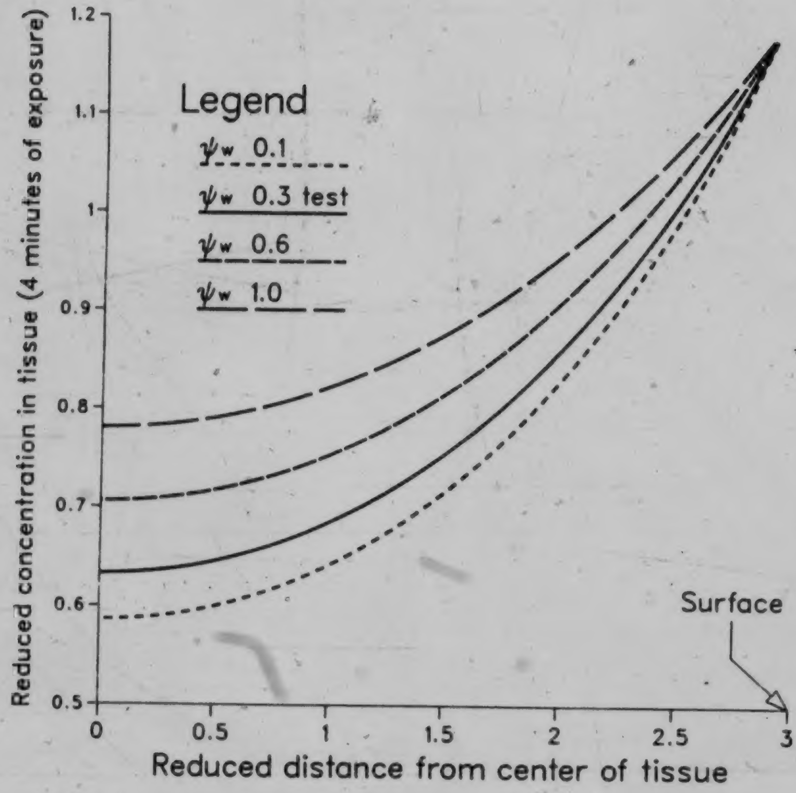

Figure 9.9 Effect of varying the diffusibility of the cell wall complex on the concentration profile of diffusing sucrose in red beet tissue.

the size of the *"cellular pools"* from which water can be drawn.

This can be seen clearly in Figures 9.10 and 9.11, where, for the purpose of the simulations, we have tested the response of red beet and potato tissues to variations in the magnitude of the above parameter. Three values were used, namely: 0.035, 0.27 and 0.42, which correspond, respectively, to the "optimum" apparent nonosmotic volume (in reduced form) found for potato tissue, red beet tissue and human granulocytes (see section 4.4). As expected, one can observe that the extent of total weight loss is directly influenced by the value ascribed to Vd, with the predicted increase in the equilibrium weight of the tissues as the nonosmotic volume increases. This effect is particularly important for the potato system (Figure 9.11), which demonstrates clearly that potato cells contain more "transferable" water than red beet cells.

In the case of cells pertaining to a cellular structure, the influence of the apparent nonosmotic volume, as to its global effect on the total weight loss to be expected from the tissue, is intimately related to the mechanical properties of the structure. This relationship is well illustrated in Figure 9.10 by the sudden drop in weight of the red beet tissue (for a system where the cells have a reduced nonosmotic volume of 0.035) as dehydration time approaches 25 minutes. This peculiar behavior prompts discussion on the last parameter to be considered in these

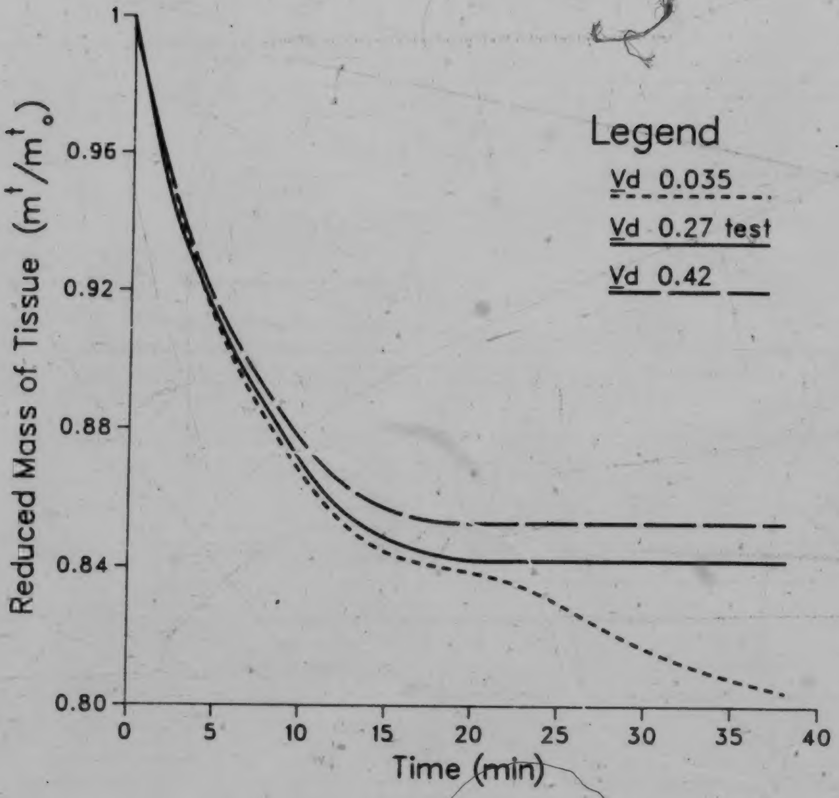

Figure 9.10 Effect of varying the apparent cellular nonosmotic volume on the course of osmotic exchanges in red beet tissue.

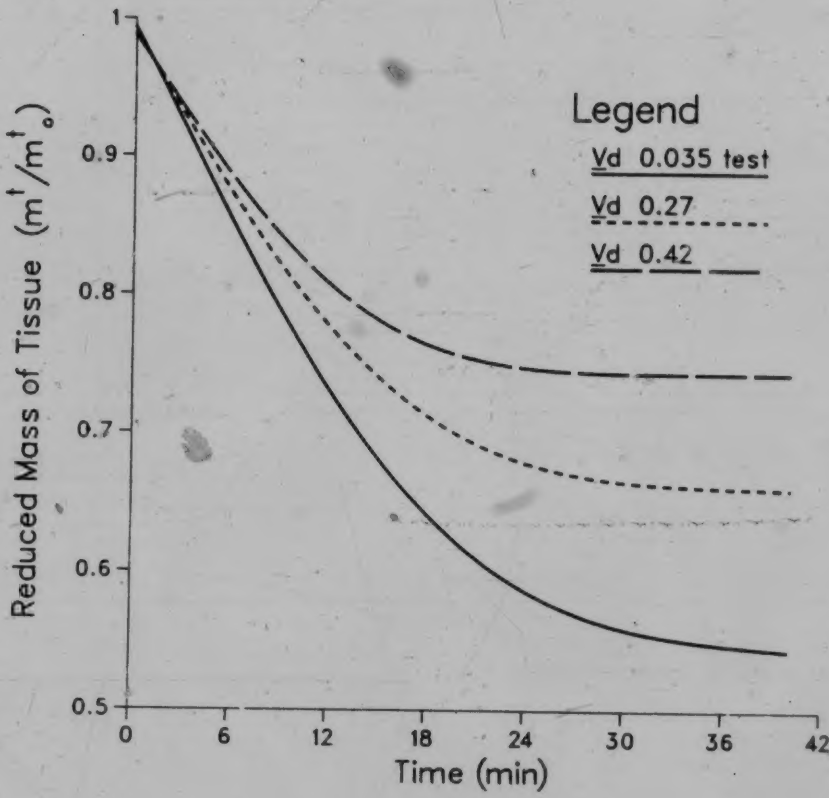

Figure 9.11 Effect of varying the apparent cellular nonosmotic volume on the course of osmotic exchanges in potato tissue.

parametric studies: the *critical cellular volume.*

Critical cellular volume

Among the parameters described so far, the *critical cellular volume* represents the sole property of the tissues which depends exclusively on the characteristics of the proposed model. Whereas the membrane permeabilities, the apoplast apparent diffusibility or the apparent cellular nonosmotic volume can or could be measured experimentally, the critical cellular volume is a pure *artifice*, which needs to be introduced to allow for the application, as part of the model, of a very crude (almost intuitive) depiction of the deformation of the tissues as they undergo osmotic dehydration (see section 8.1.1).

Let us recall that this description supposes that any unit cell may attain, during osmotic exchanges, 3 different states, each having a direct influence on the behavior of the whole structure. The most crucial in that respect is the *second state* or *second stage* of dehydration delimited on one side by the point of incipient plasmolysis and on the other side by the point where the cell reaches the critical cellular volume. During that stage it is assumed that any volume lost by the cell is simply compensated for by a proportional increase in intercellular free space volume, such that the total volume of the unit (intra- plus extra-cellular volume) remains constant (see equations 8.14 and 8.15). This implies that, contrary to stages 1 and 3, where cellular losses signify equivalent reductions in *total* volume (to simulate shrinkage), the intercellular free space can serve for a while as a "buffer" or "reservoir" in which part of the water lost by the cell can accumulate without having necessarily to migrate, as is the case in the above stages.

In terms of tissue behavior, one can realize that, depending on the limits defining the "stage 2 zone", the tissue will lose more or less weight based on the quantity of material which is allowed to remain in it during dehydration. In other words, if most of the volume lost by the cells stays locally within the tissue in the intercellular free space, this can be reflected macroscopically by no noticeable weight change for the whole structure. This is exactly what is pictured in Figure 9.10 by the curve representing the behavior of red beet tissue for cells having a nonosmotic volume of 0.035. As can be observed, the course of dehydration can be divided into 3 zones corresponding more or less to regions where the majority of the cells composing the tissue are, respectively, in stage 1 (initial losses, 0 to 12 minutes), stage 2 (*plateau* in weight change, 12 to 22 minutes) and stage 3 (increase in rate, 22 to 40 minutes). For this simulation the critical cellular volume was set (in reduced form) to 0.77, which raises the question of the determination of the magnitude of that parameter.

As discussed previously, the upper limit of the second state of dehydration, i.e. the transition between stages 1 and 2, corresponds to the point of incipient plasmolysis, that is, the point where the intracellular hydrostatic pressure vanishes. Since intracellular pressure and cellular volume are linked through functional relation involving the *cell wall elastic modulus* (see equations A4.17 and A4.18), this state of the cell can be related directly to a limiting volume, for any tissue, if one solves these relations for $\not{P}c = 0$, leading, for example, for the systems under consideration, to reduced volumes of 0.81 and 0.88 for red beet and potato tissues, respectively. On the other hand, one can readily compute the *equilibrium* volume that the cell will eventually reach for the forces involved in the system by solving the Boyle-Van't Hoff law (equation 4.48) for $\underline{V}^{(1)}\infty$, which is equivalent in the present context to $\underline{V}c\infty$. The magnitude of the critical cellular volume is therefore bounded on one hand by the cellular volume at incipient plasmolysis and, on the other hand, by the equilibrium volume of the cell.

Turning back to Figure 9.10, it then becomes easier to interpret the behavior of the red beet system as the reduced apparent nonosmotic volume is decreased from 0.42 to 0.035. Using the Boyle-Van't Hoff law, one can determine that, for these values of $\underline{V}d$, the equilibrium volumes are, respectively, 0.83 and 0.71. Since the critical cellular volume for red beet tissue has been adjusted to 0.77, it then becomes obvious that, in the case of the "0.42" curve as well as the test curve ($\underline{V}d = 0.27$, $\underline{V}c\infty = 0.78$), the system cannot attain the third stage of dehydration. By contrast, when Vd is set to 0.035, this transition, which is illustrated by the sudden increase in dehydration rate, occurs as soon as the cells reach the critical volume, forcing any further losses to leave the structure, with the ensuing decrease in total weight.

This behavior is not observable in potato tissue (see Figure 9.11), where, for reduced nonosmotic volumes of 0.42, 0.27 and 0.035, the respective equilibrium volumes of 0.53, 0.40 and 0.22 can be computed. The absence of a noticeable plateau can be explained by the relatively short period of time spent by the cells in the second stage due to the important gradients present in this system. Indeed, for a critical cellular volume of 0.80 and a volume at incipient plasmolysis of 0.88, the cells are still far from equilibrium when they cross these limits. It then follows that most of the dehydration occurs with cells in the third stage.

The effect of varying the critical cellular volume for potato tissue is seen more clearly in Figure 9.12, where, for a fixed nonosmotic volume (Vd = 0.035), values of 0.60, 0.70, 0.75 (which simulates the behavior of the structure for a value close to the red beet optimum) and 0.80 (test value) have been successively given to the parameter. As can be observed, the extent of weight loss decreases substantially as this limit is lowered. This response of the tissue indicates that mass transport in this system depends

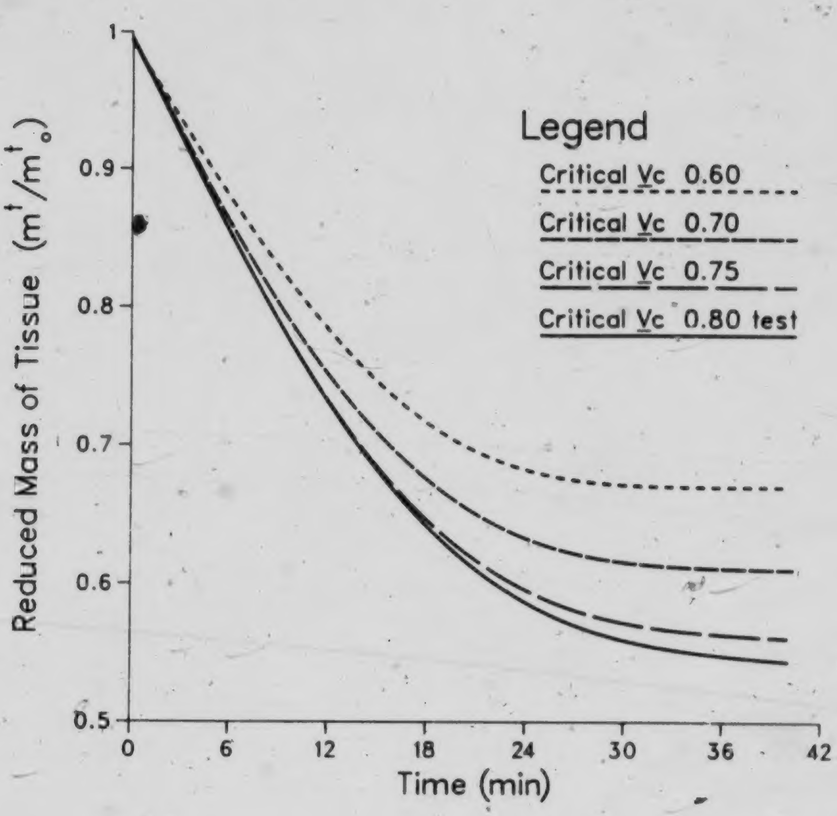

Figure 9.12 Effect of varying the critical cellular volume on the course of osmotic exchanges in potato tissue.

for a major part on the structural rearrangement occurring in the tissue as dehydration progresses. The critical cellular volume could almost be seen as a measure of the "*water holding capacity*" of the system, with a low value indicating a relative "woodenness" of the tissue, i.e. a structure which can sustain internal volume transfers, as compared to a higher value characterizing a much "softer" tissue.

9.2 Mass transfer in the presence of a permeable diffusible solute

As stated earlier, the second part of Chapter 9 will be devoted to analysing briefly results obtained when the model was used to simulate transport of a *permeable* diffusible solute in plant storage tissue. It is to be noted that the following simulations are of purely speculative nature since the results obtained cannot be compared to experimental data other than on a qualitative basis.

This study describes the behavior of a hypothetical potato disk (similar as to its characteristics to the tissue used in the "test" simulation; see section 9.1.1) in contact with osmotica containing *ethanol* and/or mannitol. Two solutes were assumed to be present in the system: mannitol, which was assimilated to the impermeable intracellular material initially occupying the tissue and was taken, if present in the osmoticum, as the *impermeable* diffusible solute, and ethanol, which acted as the *permeable* diffusible species. The following information, which is summarized in Table 9.3, was necessary to account for the latter solute:

1. *Constant* parameters. Partial molar volume and molecular weight of ethanol were obtained from Weast (1977) and were taken, respectively, as 58.36 x 10^{-3} m^3 $kgmol^{-1}$ and 46.07 kg $kgmol^{-1}$. The concentration dependence of the diffusivity of that species in water could again be described adequately by a straight-line relationship for concentrations ranging from 0 to 6M (R^2 = .9995). Coefficients a_0 and a_i of 1.240 x 10^{-5} and -0.137 were estimated (see equation 9.2), with a diffusivity at infinite dilution at 25°C of 12.40 x 10^{-10} m^2 s^{-1} (Landolt-Börnstein tables, 1969).

2. *Adjustable* parameters. Due to the permeable nature of ethanol, *6* macroscopic permeability coefficients were computed from estimates of *practical* coefficients (see section 2.2.4) characterizing the permeability properties of plant membranes for that species. No attempt was made to "adjust" these values since the predicted data could not be compared to experimental observations. These permeabilities were evaluated by solving *simplified versions* of equations 2.22, 2.23 and 2.24 (see Chartier *et al.*, 1975), in conjunction with relations 3.5 and 3.6[23], for the following *practical* coefficients: firstly, *reflection coefficients* for ethanol of 0.3 for the plasmalemma (Nobel, 1983) and 0.0 for the plasmodesma pseudo-membrane (Tyree, 1970). secondly,

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[23] Average concentrations of 55.51 (see assumption 5.2) and 1.0 kgmol m^{-3} were used, respectively, for water and ethanol.

Table 9.3 Summary of parameter values used in permeable diffusible solute study.

Description	Units	Parameter value
Osmoticum characteristics:	see Table 9.2	
- partial molar volume	m^3 $kgmol^{-1}$	
ethanol		58.36×10^{-3}
- molecular weight	kg $kgmol^{-1}$	
ethanol		46.07
- diffusivity of ethanol in water		
$D_{\blacksquare,\infty}$	m^2 s^{-1}	12.40×10^{-10}
a_0	dimensionless	1.240×10^{-5}
a_1	dimensionless	-0.137
- ethanol concentration	kgmol m^{-3}	1
Initial tissue characteristics:	see Table 9.2	
Initial cell characteristics:	see Table 9.2	
- membrane permeabilities	kgmol N^{-1} s^{-1}	
Þm( )		
water		7.00×10^{-12}
ethanol		1.18×10^{-12}
water-ethanol		6.79×10^{-13}
Þp( )		
water		1.09×10^{-9}
ethanol		3.16×10^{-10}
water-ethanol		1.41×10^{-10}

The reader is referred to the text for details on the data sources. For the sake of briefness, only the parameter values pertaining exclusively to the present studies are listed.

solute permeability coefficients of 1.12×10^{-12} kgmol N^{-1} s^{-1} for the plasmalemma and 2.96×10^{-10} kgmol N^{-1} s^{-1} for the plasmodesmata (Tyree, 1970)[24]. This led to macroscopic permeability coefficients Þm_{ik} and Þp_{ik}, for plasmalemma and plasmodesma membranes, respectively (see section 8.2.1), of 7.00×10^{-12} and 1.09×10^{-8} kgmol N^{-1} s^{-1} for *water* transfer ($i = k \equiv$ water)[25], 1.18×10^{-12} and 3.16×10^{-10} kgmol N^{-1} s^{-1} for *ethanol* transfer ($i = k \equiv$ ethanol) and 6.79×10^{-13} and 1.41×10^{-10} kgmol N^{-1} s^{-1} for the *cross-coefficients* ($i \neq k$; $i \equiv$ water and $k \equiv$ ethanol).

It should be emphasized that these values are purely for illustrative purposes since none of the practical coefficients quoted above were obtained explicitly from studies involving potato cells. In fact, these estimates characterize entirely different systems (see Tyree, 1970), since experimental estimations of permeability coefficients, for cells of higher plants in contact with such permeable solutes, are almost inexistent. These values should nevertheless represent, at least in order of magnitude, reasonable estimates, since they reflect the conclusions of Tyree (1970) showing that the symplast offers for *water* and all *small solutes* the pathway of least resistance for mass transport.

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[24] These simplified versions consist essentially in assuming that the total volume flux across any membrane can be described solely by the *water* flux, such that the 2 last terms of equation 2.22 and the 2 last terms of the numerator of equation 2.23 can be neglected. Let us note that, in view of the nature of the estimates used in the calculations, these simplifications are not too restrictive.

[25] These values correspond to the "optima" found for potato tissue in the test study reported in section 9.1.1.

In terms of the *operational parameters*, substantial alterations were necessary partly because the dehydration period in some cases was considerably reduced (as we will see below), but, most importantly, because the presence of a permeable solute in the system contributed to rendering the equations to be solved extremely stiff. Hence, the initial time increment controlling the Crank-Nicolson algorithm was decreased from 0.1 second, as utilized in the previous simulations, to 0.025 second, while limiting its maximum value to *0.5 second*. Concomitantly, the convergence criterion for the above algorithm was increased to 0.1, whereas the relative error bound on the ordinary differential equations solver had to be raised from 10^{-5} to 10^{-3} due to the increased stiffness. Let us now consider the results of simulations performed under the above conditions.

Figure 9.13 depicts the course of ethanol intake and water loss in the hypothetical tissue as a function of the time of exposure to a 1M ethanol osmoticum. As can be observed, the water losses are almost directly conpensated by equivalent ethanol gains, with the result that the overall mass of the disk stays relatively constant (note that equilibrium is reached in approximately *6 minutes*). This novel response of the tissue points out two major differences in the osmotic effect of permeable and impermeable solutes: firstly, the drastic reduction in the time for equilibration and, secondly, the poor dehydration

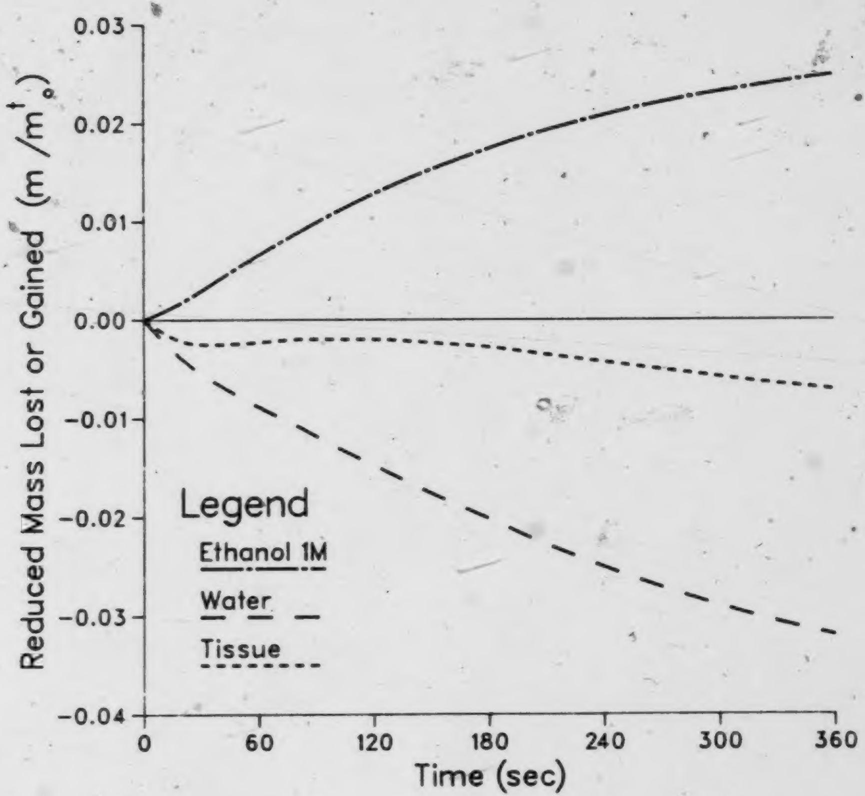

Figure 9.13 Relative ethanol intake and water loss in potato tissue as a function of the time of exposure to a 1M ethanol osmoticum.

potential of the former solutes, as can be seen clearly in Figure 9.14, which compares the response of the tissue subjected to osmotica containing permeable and/or impermeable solutes in various proportions.

Similar divergences have been reported recently by Lenart and Flink (1984), in a study on the osmotic effect of sucrose and NaCl solutions (and their mixtures) on potato tissue. Note that NaCl behaves as a *permeable* solute in plant systems, whereas it is *impermeable* for animal cells (see section 5.2.1). The authors demonstrate clearly the high penetrability of salt and its inefficacity in inducing substantial moisture losses, compared to sucrose which, although penetrating less readily, generates important water displacement. These observations, which are well in line with our findings, confirm and stress the crucial role of the membranes in the control of osmotic exchanges. Indeed, despite the fact that the higher penetrability of ethanol (or NaCl) can be explained in part by an increased diffusivity coupled with a lower concentration dependence of the coefficient, the main reason for the observed responses resides in the *effective permeability* of the membranes to these diffusible solutes which literally "opens" the whole structure to mass transfer. In that context, one cannot overemphasize the importance of the symplast in facilitating entry of the solutes into the tissue, thus contributing largely in saturating the entire system in a short period of time.

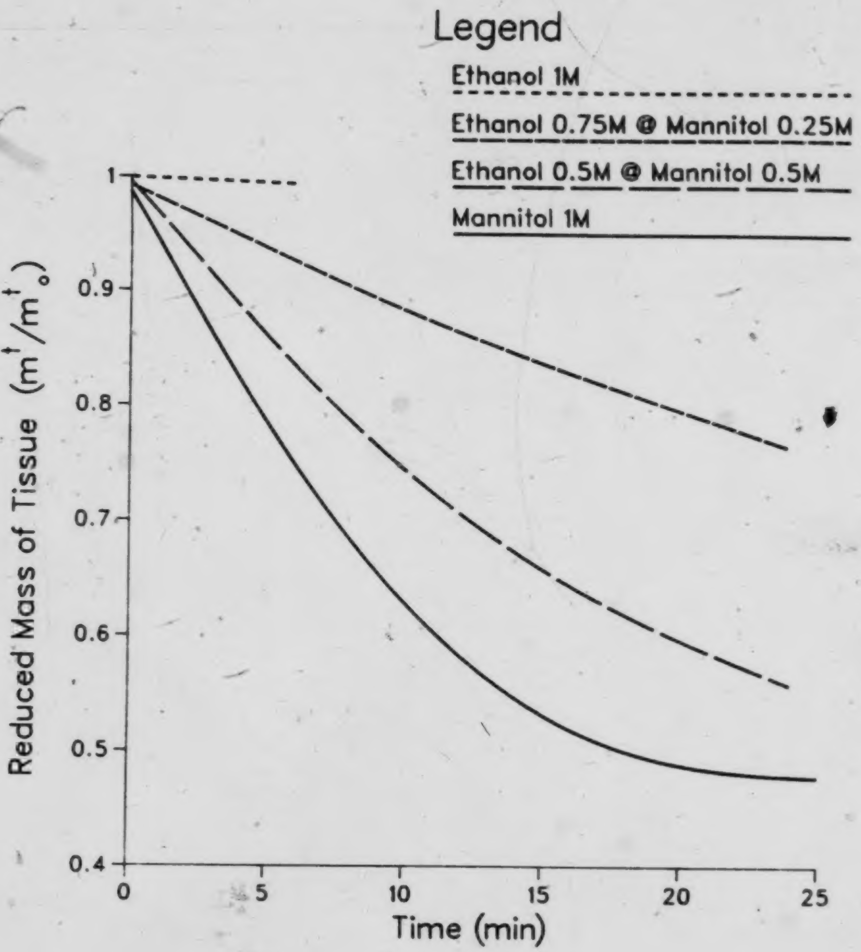

Figure 9.14 Response of potato tissue to exposure to osmotica containing permeable and/or impermeable solutes in various proportions.

As for the poor dehydration potential of ethanol and NaCl, this can be explained by referring to the behavior of a cell in contact with a hypertonic solution containing a permeable solute (see section 5.1). Initially, water is lost in response to the increased extracellular chemical potential due to the presence of the permeant solute in the surrounding solution. Meanwhile, with solute *influx*, water *returns* gradually into the cell in order to maintain osmotic equilibrium, which results ultimately in the unit regaining approximately its initial volume (see Figure 5.1). This "reentry" leads therefore, on a cellular as well as on a tissue basis, to restricting the water loss to the quantity displaced by the solute, as observed in Figure 9.13. On a more general level, it then follows that a gradual decrease (or increase) in the permeable solute content of the osmoticum should yield significant modifications in the general curvature of the osmotic weight changes, as can be seen in Figure 9.14 and as pointed out by Lenart and Flink (1984).

10. GENERAL CONCLUSIONS AND RECOMMENDATIONS

The complexity of modeling mass transfer in plant storage tissues has been considerably reduced by creating a *geometrical equivalent* of these biological structures. From a microscopic complicated tridimensional process, diffusion is now conveniently handled unidimensionally. Still, the model maintains its predictive potential since it simulates basically all the main phenomena present in the real system. For instance, by modifying judiciously the diffusivities using the *obstacle approach* (equation 8.79), the tri-dimensional complexity of the apoplast network can be simulated even if, for modeling purposes, the apoplast is approximated by a continuous cylinder. This crucial assumption is based on the fact that the parenchymatous tissue represents a rather homogeneous structure and since, as in most biological tissues, water is omnipresent, isotropy of the material can be assumed.

The main advantage of the proposed equivalent structure is that it allows each cell, through the interstitium cylinder, to experience the presence of concentration gradients in the extracellular solution all along its surface of exchange. At this level, relations derived from the theory of irreversible thermodynamics are used to take into account the selective nature of the cell membrane with regard to the transport of material across it. This approach, which is employed also for symplastic transport, results in a closer picture of mass transfer in these complex systems since it permits quantification of the resistances of these barriers to permeation.

Based on both quantitative and qualitative results obtained in this study, it is reasonable to conclude that the model proposed in this work is able to fulfil its role of describing the behavior of parenchymatous storage tissues undergoing osmotic dehydration. This conclusion relies not only on the observed good agreement between experimental and predicted data representing *macroscopic* responses of the studied structures (in the form of total weight changes), but also because these simulations were carried out using parameters which, independent of their nature, describe *microscopic* properties of the systems, the values of which comply relatively well with published literature estimates.

This is the first time, to our knowledge, that such depiction of mass transfer in complex cellular structures has been attempted in the context of osmotic dehydration involving permeable and impermeable solutes. A natural extension of the present work would be to model *animal* tissue behavior, with possible interesting applications in the domain of the prediction and control of osmotic exchanges in the pretreatment of biomaterials with cryoprotectants. Such an effort may however be premature, given the numerous assumptions used in the present work. It may be more fruitful to first concentrate one's effort to eliminate, for example, the questionable use of the *apparent cellular nonosmotic volume*, by introducing a better thermodynamical description of the forces. This could include, among other things, the *sorptional* properties of the intra- and extracellular material. Provision for handling transport of *electrolytes* would also be a logical improvement for the model, especially since extensive experimental work has been done at that level for plant and animal tissues (Poole, 1976).

Still, the most uncertain links in any analysis are the values given to the macroscopic permeability coefficients of the membranes. This dependence on reliable estimates prompts a systematic determination of those coefficients for plant and animal systems involving permeable as well as impermeable solutes, electrolytes and nonelectrolytes, coupled with temperature sensitivity studies, evaluation of the possible toxic effect of the solutes, etc. The fundamental approach to the estimation of permeability coefficients described in Chapters 4 and 5 represents therefore a powerful aid to achieving this goal. It is however important to realize that the information gained from the application of this approach is purely *phenomenological* and that any attempt to relate the observed changes to *structural* properties of the membrane is dependent on concurrent developments in the understanding of the nature and properties of the barrier at the *molecular level*.

To that respect, in terms of future investigations, it would be interesting to verify for instance if the water permeability is really influenced by the direction of flow (see section 5.2.1), or if the observed change in the value of that coefficient could be ascribed to its dependence on the osmoticum osmolality or, as reported by Diller and Bradley (1984), on the composition of the intracellular solution. Further work is undoubtedly necessary in order to clarify the question. At any rate, a precondition to the success of such a study would rely on the use of a method which permits monitoring of the behavior of the phenomenological coefficients for *small* concentration increments (or decrements), not only to assure that the system stays in the region of validity of the theory of irreversible thermodynamics (see section 2.2.1), but also to allow precise examination of the fluctuations in the values of the coefficients. This would imply, among other things, that much smaller sampling times would be necessary.

Briefly, the method could consist in exposing the studied cells (initially at isotonicity) to a slightly hypertonic (hypotonic) osmoticum, to allow them to equilibrate, then to again slightly increase the hyperosmolality (hypoosmolality) of the solution until a new equilibrium is attained, and to repeat the sequence until the entire range of osmotic values has been covered. This method would have the advantage of minimizing the osmotic stress endured by the studied cells, since they would be brought *gradually*, in a *stepwise* manner, from isotonic states to hypertonic or hypotonic environments. This *small perturbation approach* (see Farmer and Macey, 1970) could lead simultaneously to a detailed study of the apparent nonosmotic volume of the cells.

In conclusion, let us point out that, although the model presented in Chapter 8 is far from being complete (if such is in fact possible), we feel that it offers some definite possibilities which can be foreseen from the results obtained in the previous sections. With further improvements it should become a valuable research tool in the characterization of complex cellular systems.

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12. APPENDIX 1: THE SIMPLEX ALGORITHM

12.1 Introduction

The passive transport of material through membranes can be described by systems of linear equations known as *Onsager phenomenological equations* (see Chapter 2). Using the principles of *irreversible thermodynamics* one can obtain first-order differential equations for the rate of solution flow and the flow rates of different solutes across the membrane. The volume and the concentration changes of the intracellular material that occur when a cell is placed in a solution containing penetrating and nonpenetrating solutes can then be described by such a system of equations (see section 4.1.1).

On the other hand one might attempt if concentration and/or volume changes data are available to statistically fit the equations to the set of data to obtain best-fit values of the permeability coefficients contained in the Onsager equations. This evaluation has proved to be of great interest in characterizing the membrane properties and in trying to describe the mechanisms that control transport of material through membranes (Stusnick and Hurst, 1972; Hempling, 1967; Macey, 1979).

A general numerical optimization technique has thus been adopted for fitting such a system of differential equations to a given set of experimental data in order to determine best-fit values of these permeability coefficients. The method developed results not only in the best-fit coefficient values but also in estimates of the uncertainties in these coefficients. The details of this algorithm are given in this appendix.

12.1.1 Fitting of nonlinear functions to a set of data points

The best-fit values of a set of n parameters P_j in a function:

$$Y(\theta) = f(P_1,\ldots,P_n,\theta) \qquad j = 1,2,\ldots,n \qquad [A1.1]$$

which describes a set of m data points (Y_i,θ_i) are those for which the chi-square statistic:

$$\chi^2(P_1,\ldots,P_n) = \Sigma_i\{[Y_i-\hat{Y}_i]^2/\hat{\sigma}_i^2\} \qquad i = 1,2,\ldots,m \qquad [A1.2]$$

is a minimum, where $\hat{Y}_i$ is the estimated function value at θ_i for a given set of P parameters and $\hat{\sigma}_i^2$ is an unbiased estimate of the i-th sample variance or the estimate of error in the data point Y_i.

This χ^2 function will be an extremum if:

$$\partial\chi^2/\partial P_j = 0 \ ; \ j = 1,2,\ldots,n \qquad [A1.3]$$

In the case where the fitting function $f(P_i,\ldots,P_n,\theta)$ is linear in the parameters equation A1.3 leads to a system of linear algebraic relations, the *normal equations*, which can easily be solved for the best-fit values of these parameters (*linear regression*). When $f(P_1,\ldots,P_n,\theta)$ is nonlinear in the parameters, various iteration procedures, which repeatedly improve on some initial estimate of these parameters, must be used to estimate the values of the parameters which minimize χ^2 (*nonlinear regression*). Thus, expressed in the form of equation A1.2, this estimation problem appears simply as an *optimization problem* in parameter space in which the Y's and θ's are given numbers and the P's are the variables.

12.1.2 Nonlinear optimization

The tools of deterministic nonlinear optimization are numerous and the reader should particularly consult the following text for a comprehensive survey of the methods available: Gill, Murray and Wright (1981). From the user's viewpoint, these optimization techniques fall into two broad classes: (1) derivative method in which, in the search for the extremum, the partial derivatives of the fitting function with respect to each P parameters must be calculated either analytically or numerically and (2) derivative-free methods in which the evaluation of the partial derivatives is not necessary. In terms of choosing a method, problems are usually classified based on properties of the objective and constraint functions. However, even within these categories, the solution method will vary depending on the information that the user can provide about the problem functions, the available storage and the costs of evaluating the problem functions compared with the arithmetic operations performed by the algorithm. The general rule in selecting a method is to make use of as much derivative information as can reasonably be provided (Gill *et al.*, 1981).

12.1.3 Selecting a method: the Simplex algorithm

The generally accepted techniques for nonlinear parameter estimation based on least-squares minimization are derivative methods such as the Gauss-Newton method or the Levenberg-Marquardt method (Gill *et al.*, 1981). These algorithms are particularly appealing since they have been specially devised for least-squares problems. Nevertheless they require computation of derivatives either analytically or numerically. In the case where the fitting function is known, this is rather a simple matter. However, in the problem presently under consideration the fitting function is not known. Instead we know that it is one of the solutions to a set of nonlinear differential equations subject to some initial conditions. The use of these methods becomes therefore a major task since numerical integration of this system of differential equations must be carried out each time a sequence of function values is needed, not only in the estimation of the required gradients by finite differences, but as part of the arithmetic of the chosen algorithm (Stusnick and Hurst, 1972). It seems therefore obvious that in terms of computer time economy, a derivative-free method would be more adequate since it requires the least information about the fitting function (Himmelblau, 1970).

Although the selection of an optimization method remains always debatable, it appeared from a review of the available derivative-free techniques, also referred to as *sequential* or *direct-search* methods (Box, Davies and Swann, 1969; Dixon, 1972), that the *Simplex search procedure* represented a valid choice for the problem under study. The basic simplicity of the algorithm, which controls the search toward the optimum guided by calculations and decisions that are rigidly specified, makes it especially robust, and not only desirable in any procedure intended for general use but also particularly attractive for its implementation on small computers. In terms of its application to curve fitting a further advantage of the Simplex method is that the *variance-covariance matrix* of the parameter estimates is readily obtainable from the points of the final simplex configuration (Spendley, 1969). Furthermore, the features that are introduced to adapt the algorithm for least-squares optimization offer enough guarantees concerning convergence to ensure reliability since, as we will see, the implemented procedure permits a more accurate exploration of the chi-square hyperspace and consequently a better search of the extremum. A detailed description of the Simplex algorithm is presented in the next section.

12.2 The Simplex method

In order to avoid confusion, it is necessary to decide what a *"best"* response means. In optimization of an instrument, it may be a maximum response , while in fitting a function to an equation, it usually means a minimum of the sums of the squares of the differences between the experimental and the calculated data. In this treatment we will assume that a *"better"* response has a *smaller* value, as in this least-squares minimization process.

A simplex is a geometric figure in n dimensions consisting of n+1 *vertices* or *"corners"* not all in the same n-1 dimensions. Each dimension (*axis*) corresponds to a parameter or variable of the fitting function to be optimized. Thus a two-dimensional simplex is seen to be a triangle, a three-dimensional simplex is seen to be a tetrahedron, etc. The vertex coordinates are defined with respect to each of the axes so that a set of variable values is attached to all vertices.

The use of a simplex in a direct search context was first suggested by Spendley, Hext and Himsworth (1962). The currently accepted Simplex method is that due to Nelder and Mead (1965a, b). Their version of the basic simplicial approach allows for more flexibility in the simplex design and is considered as the most efficient of all current sequential techniques (Box *et al.*, 1969).

12.2.1 The Simplex method of Nelder and Mead

We will describe in the following lines the general iterative process as proposed by Nelder and Mead (1965a,b). In this variation there are three basic operations, namely *reflection*, *expansion* and *contraction*. These various steps will be illustrated with sketch diagrams for the case of a least-squares minimization search in just *two independent variables* (two-dimensional space). Then we will conclude the presentation by discussing the important questions of constrained optimization and convergence criteria.

The iterative process

Let the vertices of the simplex be V_i and the corresponding responses be F_i, i = 1,2,...,n+1. As stated previously, the response F_i is here defined as the chi-square statistic (equation A1.2) calculated by integrating numerically the system of differential equations with the i-th vertex variable values as the equation parameters.

In the process of comparing the generated function values to the experimental ones, *weighting factors* ($\hat{\sigma}_i^2$) are included in the calculation to ensure that the experimental errors are taken into account. This technique, called *weighted least-squares* (see section 4.3.3), permits a more realistic fitting (in a statistical sense) since the resulting sum of squares (chi-square statistic) is representative of the quality of experimental data (Toupin *et al.*, 1983).

Let 1, n and n+1 be respectively the subscripts of the vertices possessing the smallest, next-to-the-largest and largest responses, and let $\bar{V}$ be the centroid of all vertices excluding V_{n+1}, that is:

$$\bar{V} = (1/n)\ \Sigma_i V_i \ ;\ i = 1,2,\ldots,n \text{ and } i \neq V_{n+1} \qquad [A1.4]$$

The optimization procedure begins with the choice of the n+1 vertices and evaluation of the response at each. After evaluation of the response at each point, the vertex which gives the least desirable response, V_{n+1}, is discarded and a new vertex is chosen according to the following algorithm.

Consider the initial simplex represented by V_1-V_n V_{n+1} in Figure A1.1. Initially V_{n+1} is reflected across the face through $\bar{V}$ to give a new vertex V_r , where:

$$V_r = (1 + \alpha)\ \bar{V} - \alpha\ V_{n+1} \qquad [A1.5]$$

in which α is a positive constant termed the *reflection coefficient*. V_r is therefore on the line joining V_{n+1} and $\bar{V}$, on the side of $\bar{V}$ opposite to V_{n+1}, such that:

$$\alpha = [\ V_r\ \bar{V}\ ]/[\ \bar{V}\ V_{n+1}\ ] \qquad [A1.6]$$

where for example [V_r $\bar{V}$] denotes the distance from V_r to $\bar{V}$. There are three possibilities to be considered for the response at point V_r :

1. The response at V_r is more desirable than the response at V_1, i.e., $F_r < F_1$. This indicates movement in

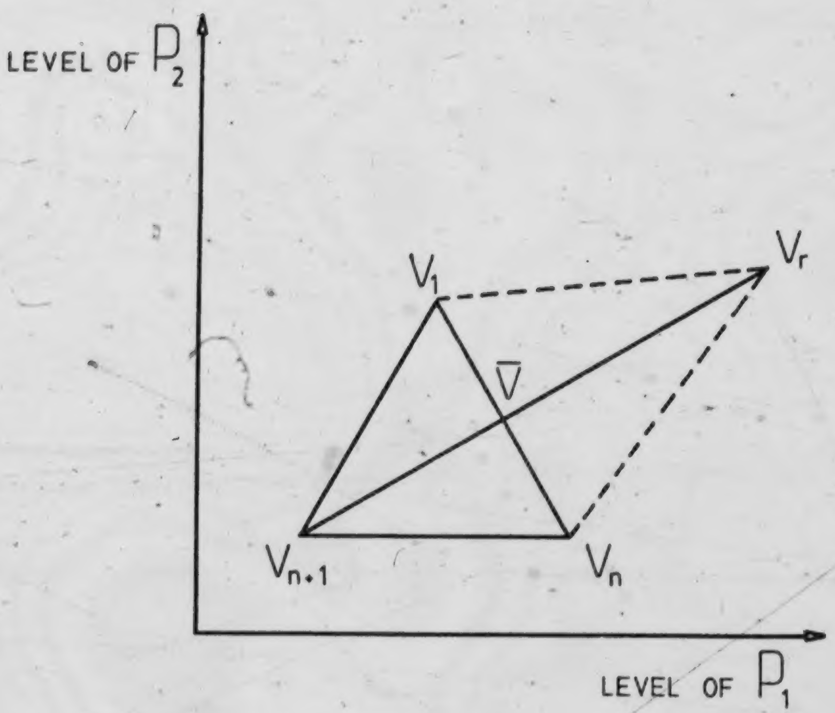

LEVEL OF P_2
V_1
V_r
$\bar{V}$
V_{n+1}
V_n
LEVEL OF P_1

Figure A1.1 Simplex search: reflection step. V_r is defined by $[V_r \bar{V}] = \alpha[\bar{V} \; V_{n+1}]$. P_1 and P_2 are the independent variables (parameters).

the proper direction and suggests further investigation. Therefore a new point V_e , on the extended line $V_{n+1}\ \bar{V}\ V_r$ is calculated, where:

$$V_e = \gamma\ V_r + (1 - \gamma)\ \bar{V} \qquad [A1.7]$$

in which the expansion coefficient, γ, is given by:

$$\gamma = [\ V_e\ \bar{V}\ ]/[\ V_r\ \bar{V}\ ] > 1 \qquad [A1.8]$$

If the response at V_e is more desirable than the response at V_r , i.e., $F_e < F_r$, then the expansion has been successful and V_{n+1} is replaced by V_e , the new simplex is $V_1\ \ V_n\ \ V_e$. If F_e is not more desirable than F_r, the expansion is considered a failure, and V_{n+1} is replaced by V_r . The new simplex is $V_1\ V_n\ V_r$.

A *failed expansion* can be seen as resulting from a lucky foray of the reflected point into an area of small response values but at an angle such that the expansion process brings the generated vertex back into a surrounding region of higher responses. For the purpose of illustration, the reader is referred to Figure A1.6, case B.

2. The response at V_r is neither better than the response at V_1 nor worse than the response at V_n, that is, $F_1 \leq F_r \leq F_n$. Since neither expansion nor contraction is clearly indicated, the new simplex is $V_1\ V_n\ V_r$.

3. The response at V_r is less desirable than the response at V_n, i.e., $F_r > F_n$. This indicates unsatisfactory movement and suggests contraction in this particular

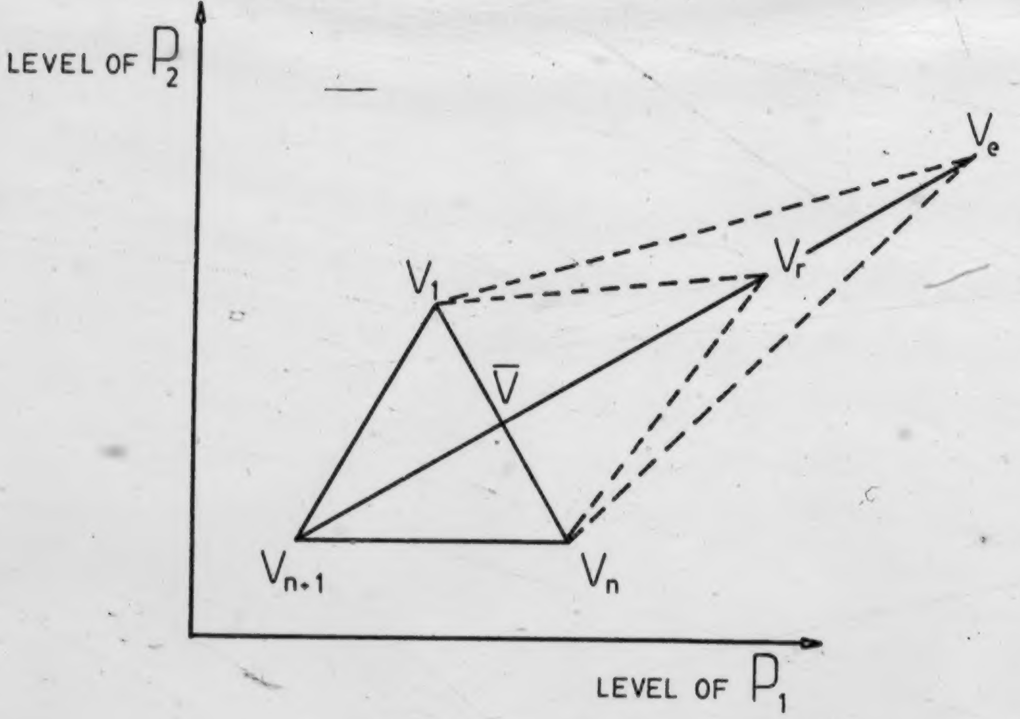

LEVEL OF P_2
LEVEL OF P_1
V_e
V_r
V_1
$\bar{V}$
V_{n+1}
V_n

Figure A1.2 Simplex search: expansion step. V_e is defined by $[V_e\bar{V}] = \gamma[V_r\bar{V}]$.

direction of investigation.

Case (i): If the response at V_r is less desirable than the response at the worst previous vertex (point V_{n+1}), then the new contracted simplex should lie at V_C which is closer to V_{n+1} than V_r, where:

$$V_C = \beta V_{n+1} + (1 - \beta) \bar{V} \qquad [A1.9]$$

in which the *contraction coefficient* β is given by:

$$\beta = [V_C \bar{V}]/[V_{n+1} \bar{V}] \; ; \; 0 < \beta < 1 \qquad [A1.10]$$

If $F_C < F_{n+1}$, the contraction is considered successful, V_C replaces V_{n+1}, the new simplex is V_1 V_n V_C and the basic process is recommenced.

Case (ii): If the response at V_r is somewhat more desirable than the response at the worst previous vertex, i.e., $F_r < F_{n+1}$, then the new contracted simplex should lie at V'_C which is closer to V_r than V_{n+1}, where:

$$V'_C = \beta V_r + (1 - \beta) \bar{V} \qquad [A1.11]$$

in which β is, as before, the *contraction coefficient*. Note that in this case V_r replaces V_{n+1} as the worst vertex in equation A1.9 to yield equation A1.11.

If $F'_C < F_r$ the contraction step is successful, V_r is then replaced by V'_C, forming the new simplex V_1 V_n V'_C and the search process is restarted. If V_C or V'_C should prove to be the least desirable vertex in the new simplex (*failed contraction*), corrective action can be taken to assure that

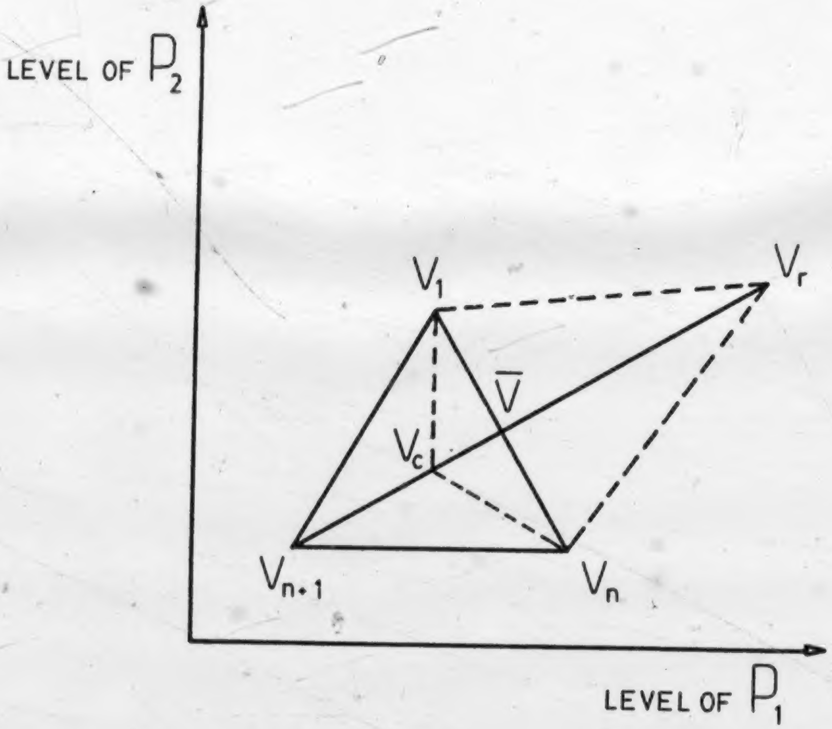

LEVEL OF P_2
LEVEL OF P_1
V_1
V_r
$\bar{V}$
V_c
V_{n+1}
V_n

Figure A1.3 Simplex search: contraction step [case (i)]. A contraction step is called for if $F_r > F_n$. If $F_r \geq F_{n+1}$ then V_c is defined by $[V_c \bar{V}] = \beta[V_{n+1} \bar{V}]$.

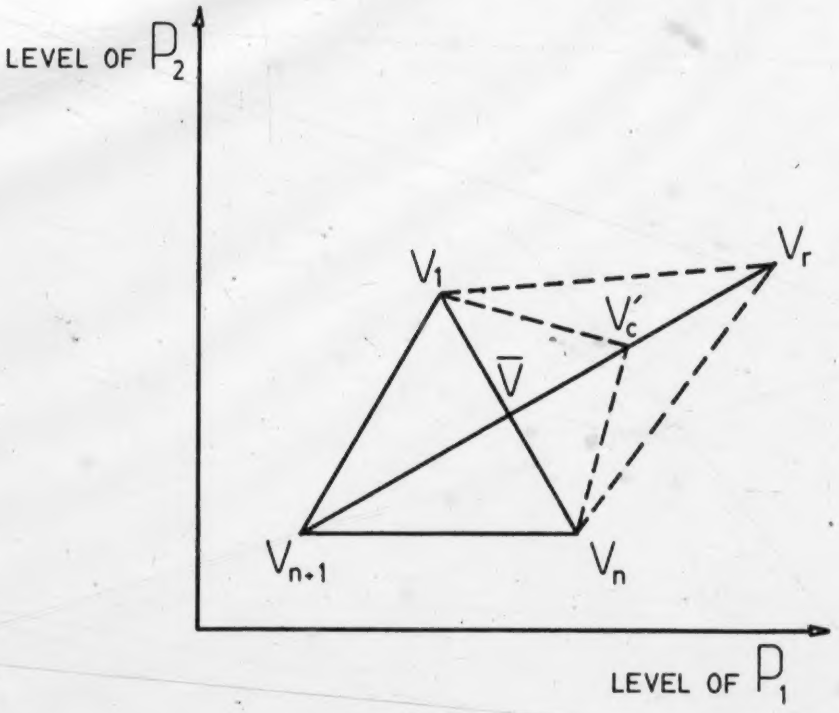

LEVEL OF P_2
LEVEL OF P_1
V_1
V_r
V_c'
$\bar{V}$
V_{n+1}
V_n

Figure A1.4 Simplex search: contraction step [case (ii)]. A contraction step is called for if $F_r > F_n$. If $F_r < F_{n+1}$, then V_r replace V_{n+1} and V_c' is defined by $[V_c'\bar{V}] = \beta[V_r\bar{V}]$.

the simplex does not become stranded. Nelder and Mead (1965a) suggest that a new configuration be set up by halving the distance from V_1 of all the vertices of the simplex before the basic process is continued. This *shrinkage process* could also be used if the best point remains unchanged for many iterations (note that the best point may change during the shrinking process). This operation can be generalized by introducing the *shrinkage coefficient* δ (Parkinson and Hutchinson, 1972) so that:

$$V'_i = \delta\, V_i + (1 - \delta)\, V_1 \qquad [A1.12]$$

in which δ takes normally the value 0.5 and V'_i is the i-th shrunk vertex.

A failed contraction is seldom encountered, but can occur when a "*valley*" (area of small response values) is curved and one point of the simplex is much farther from the valley bottom compared to others, i.e. has a much higher response. Let us consider for example the two-dimensional space represented by the following "*landscape*". The curved lines correspond to lines of equal response value and the numbers represent their relative levels as compared to the "*valley*" response. Let us imagine that at a given stage of the search (case A), the simplex is defined as V_1 V_n V_{n+1}, where the subscripts have the same meaning as before. The reflection operation generates V_r with response $F_r = 23$, where 23 means that the response is located between level 2 and 3. Since $F_r > F_n$ ($F_n = 12$), a contraction is called for

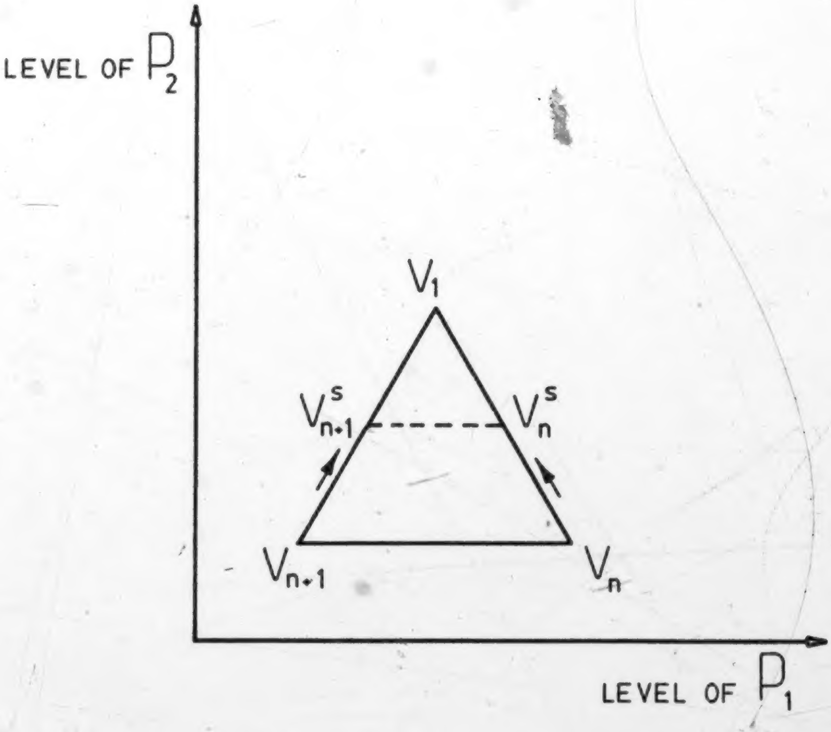

LEVEL OF P_2
V_1
V_{n+1}^s
V_n^s
V_{n+1}
V_n
LEVEL OF P_1

Figure A1.5 Simplex search: shrinkage process. All the vertices are shrunk toward the best vertex, V_1, then V_i^s is defined by $[V_i^s V_1] = \delta[V_i V_1]$, where $i \neq 1$.

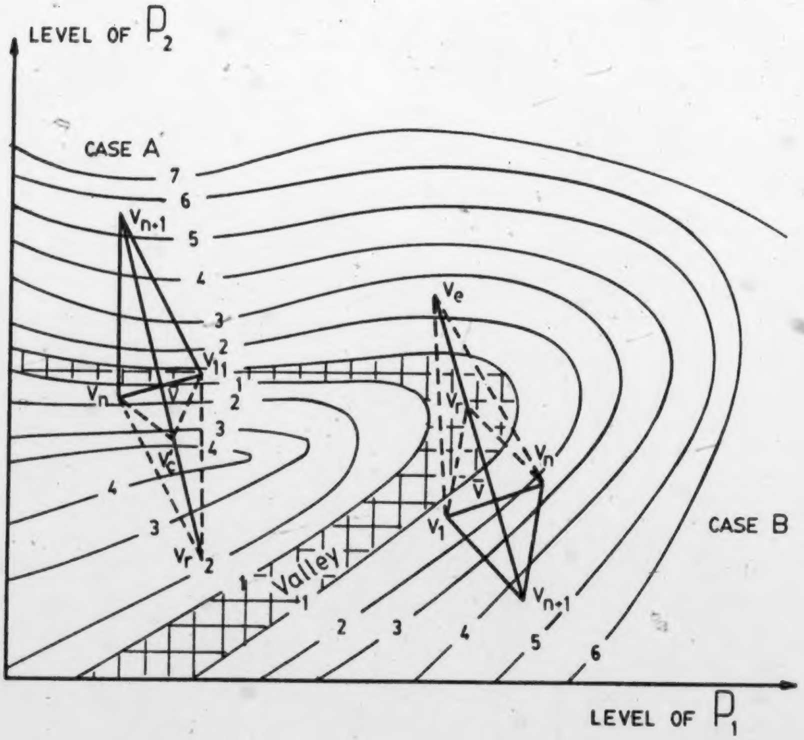

LEVEL OF P_2
CASE A
7
6
5
4
3
2
1
V_{n+1}
V_1
V_n
$\bar{V}$
V_c
V_r
4
3
2
Valley
V_e
V_r
V_n
$\bar{V}$
V_1
V_{n+1}
CASE B
1
2
3
4
5
6
LEVEL OF P_1

Figure A1.6 Simplex search: illustration of failed contraction (A) and failed expansion (B); see text for details.

and since $F_r < F_{n+1}$ ($F_{n+1} = 56$), the trial vertex V'_c should lie between $\bar{V}$ and V_r . As can be seen, the operation is unsuccessful since V'_c, being now located on a ridge, has the least desirable response of the new simplex, that is , $F'_c > F_r$ ($F'_c = 34$). If, as in the expansion process, V_{n+1} was then replaced by the reflected point V_r , it is easy to see that the simplex would become stranded since for the new simplex V_n V_1 V_r the reflected point is V_{n+1}. Contractions would consequently be called for and V'_c always generated making these operations useless. That is why Nelder and Mead proposed the shrinkage process in order to modify drastically the simplex configuration and to bring eventually by this massive contraction all the points into a better "*environment*".

It should be noted nevertheless that this example is purely illustrative and that depending on the respective values taken by the reflection, expansion and contraction coefficients, the behavior of the simplex will be altered and will consequently yield a different surface exploration.

The choice of the best search strategy

The choice of the values of the *operational coefficients* α, β and γ is termed a *search stategy*. In their study Nelder and Mead (1965a) found that useful values were $\alpha = 1$, $\beta = 0.5$ and $\gamma = 2$, corresponding to a simple reflection, halving when in difficulty and doubling when a useful direction is located. Parkinson and Hutchinson (1972) on the other hand found that the set $\alpha = 2$, $\beta = 0.25$ and $\gamma =$

2.5 was somewhat superior, but stated that this could be partly due to the nature of the test functions used in their study. Nevertheless, they suggested that the set $\alpha = 1$, $\beta = 0.25$ and $\gamma = 2.5$ could be used as a safe global strategy giving typical gains of about 10%. The latter operational coefficients were then selected in view of this possible gain but particularly because it permitted to elegantly generalize the original reflection, expansion and contraction relations (equations A1.5-A1.11) as proposed by Deming and Morgan (1973).

Indeed, since the reflection coefficient is unity, equation A1.5 can be rewritten as:

$$V_r = 2\,\bar{V} - V_{n+1} \qquad [A1.13]$$

Replacing V_r in equation A1.7 by the above relation then yields:

$$V_e = \bar{V} + \gamma\,(\bar{V} - V_{n+1}) \qquad [A1.14]$$

Similarly, equation A1.11 becomes:

$$V_c' = \bar{V} + \beta\,(\bar{V} - V_{n+1}) \qquad [A1.15]$$

Equations A1.5 and A1.9 can also be transformed along the same lines to give respectively:

$$V_r = \bar{V} + \alpha\,(\bar{V} - V_{n+1}) \qquad [A1.16]$$

and

$$V_c = \bar{V} - \beta\,(\bar{V} - V_{n+1}) \qquad [A1.17]$$

As we can see, a general equation emerges from the previous ones. This relation is only a function of the centroid and the worst vertex to be discarded and takes the form:

$$V_{(\ \ )} = \bar{V} + \lambda\ (\bar{V} - V_{n+1}) \qquad [A1.18]$$

where $V_{(\ \ )}$ is the newly generated vertex and λ is the *operational coefficient* taking the value of 1 for a reflection operation, 2.5 for an expansion and -0.25 or 0.25 for a contraction process corresponding to case (i) or case (ii) respectively.

12.2.2 Constrained search and the Simplex algorithm

The Simplex technique described previously can be further modified for use with inequality constraints of the type $h_k(P_1,...,P_n) \leq 0$, k= 1,2,...,t; where h_k is one of the t constraint relations, a function of the model parameters (Dixon, 1972). Constraints or boundaries can be seen as the limits of regions in the n-dimensional space where the parameters take values that have either no physical significance or are simply not acceptable. The algorithm must consequently, counteract any incursion in these regions by forcing the search to resume in an area of valid parameter values.

This can be easily performed in the Simplex procedure by giving to the vertex that contravenes the constraints, a very undesirable response value much larger than the response of the actual worst vertex. The search is then automatically directed away from the boundaries either by a contraction or a massive shrinkage operation. Figure A1.7 illustrates the process.

Let us imagine that at a given stage of the search, the simplex is defined as V_1 V_n V_{n+1}, where the subscripts have the same meaning as before. The reflection generates V_r which, as can be seen, violates the limit imposed on P_1 parameter values. A very undesirable response is then assigned to V_r such that $F_r >> F_{n+1}$. Since $F_r > F_{n+1} > F_n$, a contraction is automatically called for, which, if successful, will generate V_1 V_C V_n or if not, will generate the shrunk simplex V_1 V^*_{n+1} $\bar{V}$. In either case the process has thus forced the search to stay in a valid region of parameter values.

In the very unlikely situation where a massive shrinkage should bring one of the vertex in a forbidden region, we propose that the algorithm simply restores the vertex to its initial position. Since it is very improbable that all the other vertices will undergo the same process, the simplex configuration will be sufficiently altered to ensure that it will not become stranded on the next move. Let us note finally that numerous consecutive boundary violations must be regarded as indicative of a serious problem in the search process most probably due, as we will discuss later, to either an inappropriate initialization or to a defect in the formulation of the fitted model.

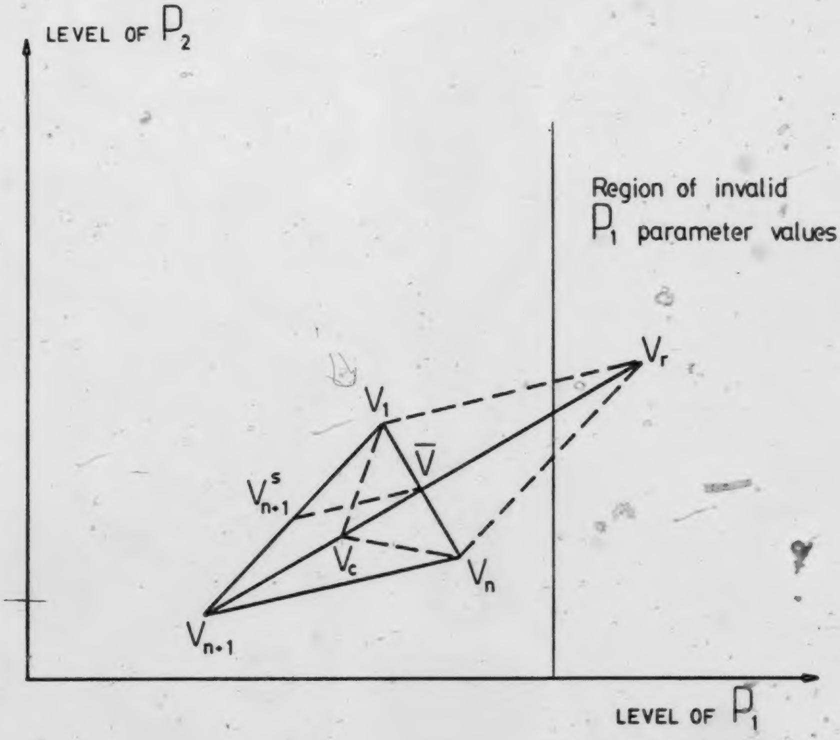

LEVEL OF P_2
Region of invalid
P_1 parameter values.
V_r
V_1
$\bar{V}$
V^s_{n+1}
V_c
V_n
V_{n+1}
LEVEL OF P_1

Figure A1.7 Constrained Simplex search: boundary violation.

12.2.3 Convergence criteria

A crucial point concerns the criteria used for halting the procedure. It is clear that in general, terminating criteria do not guarantee convergence, and that different criteria will cause termination at different positions and for different response values.

The simplest terminating criteria are based on corrections becoming sufficiently small, gradients becoming small, response values becoming close, or, as employed in this study, a quadratic fit giving an "*interior*" minimum. Combinations of these are frequently employed, and they can also be associated with other restrictions.

Nelder and Mead (1965a) used the condition that the *standard deviation* of the responses over the simplex is small, that is:

$$SD = \{[\Sigma_i (F_i - \bar{F})^2]/n\}^{1/2} \leq \epsilon \; ; \; i = 1,2,...,n+1 \qquad [A1.19]$$

where ϵ is a pre-set value and $\bar{F}$ is the average value of the simplex responses. The test is performed after *each* iteration. If the standard deviation is larger than the pre-set value, a further move is initiated, otherwise the procedure has converged and the best parameters are taken as the values of the coordinates at the best vertex (V_1) at which the response is lowest. However, as noted by the authors, the success of the criterion depends on the simplex not becoming too small in relation to the curvature of the surface until the final minimum is reached. A convergence on a *local minimum* is therefore always possible since, in that case, the standard deviation could be sufficiently small to comply with the test.

Hence, Box *et al.* (1969) suggested that a possible improvement on this test could involve the calculation of SD after every k iterations, k being pre-assigned. The requirements would then be that two successive values of SD were less than the specified value ϵ, and that the corresponding mean values $\bar{F}$ did not differ by more than some specified amount. Both criteria would ensure that the search continued until the simplex had virtually collapsed onto the indicated minimum. Other procedures have also been suggested for convergence tests (Parkinson and Hutchinson, 1978; Deming and Morgan, 1973; Ryan, Barr and Todd, 1980), nevertheless, due to the present use of the Simplex algorithm for nonlinear least-squares minimization, the approach found most satisfactory is somewhat different from the preceding ones.

Quadratic fit as convergence criterion

The procedure used to detect convergence in the study under consideration is principally based on the quadratic fit that is performed as a necessary step in obtaining the variance-covariance matrix of the parameter estimates. Since this topic will be covered in more details in the next section, we will restrict the following discussion in presenting only the main features of the convergence procedure.

The convergence criteria can be divided in two main parts. Firstly, tests are applied at the level of each generated vertex. Thus, after an iteration, the *coefficient of variation* (CV) of the responses over the simplex is calculated, that is (Steel and Torrie, 1980):

$$CV = SD / \bar{F} \leq \epsilon_1 \qquad [A1.20]$$

Concurrently, the *relative difference* (RD) between the average response of the previous simplex and the average response of the current one (k-th simplex) is estimated, that is:

$$RD = |(\bar{F}_{k-1}-\bar{F}_k)/\bar{F}_k| \leq \epsilon_2 \qquad [A1.21]$$

The first criterion, similar to Nelder and Mead's, tests for a possible convergence on a minimum while the second one, by restricting the range in $\bar{F}$ values, ensures that the search will effectively locate a *true minimum* since as the simplices approach the neighbourhood of a *global extremum*, the corrections to $\bar{F}$ values will become necessarily small. If at that stage the values of CV and RD are respectively larger than ϵ_1 or ϵ_2, a further move is performed, otherwise the search is stopped.

The advantage in using *relative values* as stopping criteria is that they do not require any knowledge of the expected *absolute values* of either the standard deviation or the responses which, as we know, can vary substantially from problem to problem. They are therefore more "*meaningful*" to the user since they represent basically *percentages of deviation* from an acceptable target.

Once a minimum is located, the second part of the halting procedure begins. A quadratic fit is performed using the latest simplex responses (see next section for details). An "*internal*" minimum is then obtained in conjunction with an estimate of the variance-covariance matrix at that minimum. As we will see, this matrix is related to the *Hessian matrix* often called the *information matrix* from which the character of the stationary point can be studied. If it is found that this quadratic optimum is a true minimum the whole search has converged, the parameters are then taken as the coordinates of this internal minimum. On the other hand, if the study of the elements of the Hessian matrix shows that this optimum is not a true minimum, a new simplex is then generated using the coordinates of the previous best vertex as initial values and a further exploration of the hyperspace is initiated with the test coefficients ϵ_1 and ϵ_2 decreased by a factor of ten.

This process continues until a true minimum is found or until the test coefficient ϵ_1 reaches a value which is of the same order of magnitude as the relative rounding error attached to the evaluation of any response. This latter point will be covered in section 12.5.3.

12.3 Quadratic fitting and the Simplex algorithm

Irrespective of the actual approach to a numerical search problem, the end result is the definition of the desired optimum within a given degree of accuracy. This is particularly true, if further inferences are to be drawn from the optimized parameters. In that case not only accurate estimates are necessary but the uncertainties in these parameters must also be known. Indeed, the "*true*" value of a process variable is in one sense intrinsically a hypothetical value which is postulated as existing. Tied in with this concept, which is fundamental in the description of any stochastic phenomena, is the concept of error or uncertainty, the "*error*" representing the difference between the actual estimate and the unknown true value. Thus, in the implementation of the actual algorithm, it was essential to include as part of the procedure, a method to obtain some measure of the dispersion of the parameter estimates.

12.3.1 The role of the quadratic approximation

Although the search toward an optimum can be handled quite efficiently by the Simplex algorithm, its basic weakness, which is shared by all derivative-free methods, is that when a near-stationary value of the fitting function has been found, there is absolutely no information available as to the "*nature*" of the hyperspace in the neighbourhood of that point. This is particularly limiting since the lack of definition prevents the search from being guided quickly to a conclusion. Instead, it oscillates or cycles around the true optimum. This curvature information is obviously provided by the missing derivatives and the desirable next step is therefore the fitting of some model to the responses to enable the optimum to be located more accurately (Beveridge and Schechter, 1970).

The procedure adopted in the present study was put forward by Spendley and coworkers in 1962. It is based on the assumption that close to the stationary point, the response behavior can be defined by a function which consists of the terms up to second order in the Taylor series expansion around the current optimum . The device is of special value since the form of the approximating quadratic model permits not only to locate the true optimum but gives directly the variances and covariances of the final parameter estimates.

12.3.2 Final steps in the location of the optimum

Let us consider the fitting of a quadratic surface to the responses produced in the final stage of a Simplex search. In order to estimate the .5(n+1)(n+2) constants necessary to define the quadratic approximation, n being again the number of paramaters to be optimized, one needs to generate a further .5n(n+1) response values given the n+1 responses already contained in any of the simplices. Hence selecting preferably the final simplex to provide the latter responses, Spendley *et al.* (1962) suggest that the additional vertices be taken at the mid-points of the edges of the chosen simplex, so that the locations are:

$$V_{ij} = .5\ (V_i + V_j)\ ;\ i \neq j \qquad [A1.22]$$

If the original points of the reference simplex are then used to define a set of *unit* oblique axes with coordinate vector $\mathbf{x}_i$, i= 1,2,...,n+1, these vertices may be written as:

$V_1 \Rightarrow 0\ 0\ 0\ \ldots\ 0 : \mathbf{x}_1$

$V_2 \Rightarrow 1\ 0\ 0\ \ldots\ 0 : \mathbf{x}_2$

$V_3 \Rightarrow 0\ 1\ 0\ \ldots\ 0 : \mathbf{x}_3$

....

$V_{n+1} \Rightarrow 0\ 0\ 0\ \ldots\ 1 : \mathbf{x}_{n+1}$

so that the additional *"half-way"* points are:

$V_{12} \Rightarrow .5\ \ 0\ 0\ \ldots\ 0 : \mathbf{x}_{12}$

$V_{23} \Rightarrow .5\ .5\ 0\ \ldots\ 0 : \mathbf{x}_{23}$

....

or in a generalized vectorial form:

$$\mathbf{v}_i = \mathbf{v}_1 + \mathbf{Q}\ \mathbf{x}_i \qquad [A1.23]$$

where $\mathbf{v}_i$ and $\mathbf{v}_1$ denote the coordinate vector respectively of V_i and V_1 in the *original system*, V_1 being the origin vertex usually chosen as the point giving, in the final simplex, the best response, and $\mathbf{Q}$ is the nxn transformation matrix whose i-th column is $\mathbf{v}_i - \mathbf{v}_1$.

Let the responses corresponding to each vertex be denoted by F_i or F_{ij}. Then the desired quadratic approximation will be, in *vector form*:

$$F(\mathbf{x}) = a_0 + \mathbf{a}' \mathbf{x} + .5\, \mathbf{x}' \mathbf{B}\, \mathbf{x} \qquad [A1.24]$$

where a_0, **a** and **B** are respectively a constant scalar, vector and square matrix, functions of the F_i and F_{ij} responses. Thus, assuming that $F(\mathbf{x})$ is *differentiable* at the unknown true optimum, i.e., that the partial derivatives of F with respect to each coordinate axis are continuous at $\mathbf{x*}$ (in terms of the new coordinate system), one generates the n-vector of these partial derivatives termed the *gradient vector* of F and denoted $\nabla F(\mathbf{x})$, namely:

$$\begin{aligned} \nabla F(\mathbf{x*}) &= [\ \partial F(\mathbf{x*})/\partial x_1, \ldots, \partial F(\mathbf{x*})/\partial x_n\ ] \\ &= \mathbf{a} + \mathbf{B}\, \mathbf{x*} \end{aligned} \qquad [A1.25]$$

The location of the optimum can then be determined, since by definition at that point, the gradient vector of F is null, that is all partial derivatives are zero, namely:

$$\nabla F(\mathbf{x*}) = 0 = \mathbf{a} + \mathbf{B}\, \mathbf{x*} \qquad [A1.26]$$

leading finally to the coordinates of the stationary value given by (in the new coordinate system):

$$\mathbf{x*} = -\, \mathbf{B}^{-1}\, \mathbf{a} \qquad [A1.27]$$

At this stage, it remains only to translate this location back into the original coordinates by using relation A1.23, that is vectorially:

$$\mathbf{v}^* = \mathbf{v}_1 + \mathbf{Q}\, \mathbf{x}^* \qquad [A1.28]$$

The *predicted response value* at the stationary point is then estimated to be (from equation A1.24):

$$F(\mathbf{x}^*) = a_0 + \mathbf{a}'\, \mathbf{x}^* + .5\, \mathbf{x}^{*\prime}\, \mathbf{B}\, \mathbf{x}^* \qquad [A1.29a]$$

which yields, by making use of equation A1.27 and after proper manipulations, to:

$$F(\mathbf{x}^*) = a_0 - .5\, \mathbf{a}'\, \mathbf{B}^{-1}\, \mathbf{a} \qquad [A1.29b]$$

since, as we will see, **B** is a square *symmetric* matrix.

12.3.3 The evaluation of the model constants

Estimation of a and B

In order to use equations A1.27 and A1.29b to locate the true optimum and to estimate its response value, it is necessary, as we have seen, to evaluate the constants a_0, **a** and **B** present in the quadratic model. This can be performed quite easily by taking advantage of the properties of quadratic functions (Gill *et al.*, 1981) and of the special nature of the new system of coordinates. Indeed, differentiating equation A1.24 at the *origin vertex*, i.e. at $\mathbf{x}_1$, the gradient vector of F is, as before, given by:

$$\begin{aligned}\nabla F(\mathbf{x}_1) &= [\ \partial F(\mathbf{x}_1)/\partial x_1, \ldots, \partial F(\mathbf{x}_1)/\partial x_n\ ] \\ &= \mathbf{a} + \mathbf{B}\, \mathbf{x}_1 \qquad [A1.30a]\end{aligned}$$

which can be conveniently simplified to:

$$\nabla F(\mathbf{x}_1) = \mathbf{a} \tag{A1.30b}$$

since $\mathbf{x}_1$ is a *null vector* in the new coordinate system. Similarly the n^2 "*second-partial derivatives*" can be derived and represented by a square, symmetric matrix termed the *Hessian matrix* of $F(\mathbf{x})$, which will be denoted $\nabla^2 F(\mathbf{x})$, that is:

$$\nabla^2 F(\mathbf{x}_1) = \begin{bmatrix} \partial^2 F(\mathbf{x}_1)/\partial x_1^2, \ldots, \partial^2 F(\mathbf{x}_1)/\partial x_1 \partial x_n \\ \vdots \qquad\qquad\qquad \vdots \\ \partial^2 F(\mathbf{x}_1)/\partial x_1 \partial x_n, \ldots, \partial^2 F(\mathbf{x}_1)/\partial x_n^2 \end{bmatrix} = \mathbf{B} \tag{A1.31}$$

As can be noticed, the vector a and the matrix **B** can be readily assembled if the values of the respective first and second-order partial derivatives can be computed at $\mathbf{x}_1$. In the present situation though, we must rely on numerical analysis to estimate these derivatives since analytical solutions are not available. This is carried out by making use of the principles of *finite-difference approximations to derivatives* and we refer the interested reader to the following authors for more details: Lapidus and Pinder (1982); Gill *et al*. (1981).

Finite-difference approximations to the derivatives

The choice of an approximating formula and consequently the order of its associated truncation error are directly dependent on the amount of information available around the point at which the derivatives are to be approximated. In the problem under study, the derivatives are evaluated at $\mathbf{x}_1$, the origin vertex. All other vertices lie, by reason of the transformation used, on the "*positive*" side of it, i.e., there are no negative coordinate values in any $\mathbf{x}_i$ or $\mathbf{x}_{ij}$ vectors. Consequently *forward type formulae* should be used since they only require information from points reached by making steps along the axes in a *positive direction* from the origin. The partial derivatives can therefore be estimated using the following finite-difference relations:

1. Finite-difference approximations in one dependent variable, order of error $O(h^2)$.

Case (i): First-order derivatives:

$$\partial U(z)/\partial z|_r \cong [-U_{(r+2h)}+4U_{(r+h)}-3U_{(r)}]/2h \tag{A1.32}$$

where the derivative is evaluated at point r with respect to the dependent variable z, $U_{(k)}$ is the response or function value at the k-th point and h is the step size along the z axis ($h > 0$).

Case (ii): Second-order derivatives:

$$\partial^2 U(z)/\partial z^2|_r \cong [U_{(r+2h)}-2U_{(r+h)}+U_{(r)}]/h^2 \tag{A1.33}$$

2. Finite-difference approximation in two dependent variables, order of error $O(h^2)$. Second-order derivatives:

$$\partial^2 U(z_1,z_2)/\partial z_1 \partial z_2|_r \cong$$

$$[U_{(r+h_1+h_2)}-U_{(r+h_1)}-U_{(r+h_2)}+U_{(r)}]/h_1h_2 \tag{A1.34}$$

where h_1 and h_2 are respectively the step size along the z_1 and z_2 axes (h_1, $h_2 > 0$).

Applied to our system, the above equations are rewritten as follows. Selecting the step size to be in all directions equal to 0.5 (this corresponds to the smallest distance between two points in the new system of coordinates) equations A1.32, A1.33 and A1.34 become, respectively:

$$\partial F(\mathbf{x})/\partial x_i|_{x_1} \cong [-F_i+4F_{1i}-3F_1] \qquad i = 2,3,\ldots,n+1 \qquad [A1.35]$$

$$\partial^2 F(\mathbf{x})/\partial x_i^2|_{x_1} \cong 4[F_i-2F_{1i}+F_1] \qquad i = 2,3,\ldots,n+1 \qquad [A1.36]$$

and

$$\partial^2 F(\mathbf{x})/\partial x_i \partial x_j|_{x_1} \cong 4[F_{ij}-F_{1i}-F_{1j}+F_1] \qquad i,j = 2,3,\ldots,n+1 \text{ and } i \neq j \qquad [A1.37]$$

where F_i and F_{ij} are, as before, the responses at the i-th and ij-th vertex. The role of the "*half-points*" is now manifest, since, as it is shown, they are *explicitly* part of the approximating formulae.

Spendley (1969) has proposed that in the special case of least-squares minimization, *function values* at only *n+1* points (the points corresponding to the vertices of any simplex) could be used for the same purpose to fit a quadratic model to terminate the search. Although quite appealing at first sight since it decreases the amount of information needed to estimate the derivatives, it is nevertheless hardly applicable in the present case where the *fitting function* is not known. Indeed, this approach is based on the linearization near the optimum of the fitting function in a truncated Taylor series, similar to the Gauss-Newton algorithm, and, as such, requires a sequence of numerical integrations which is economically not justified. For this reason and since the use of finite-difference approximations with lower order of truncation error would yield to substantial uncertainty in the derivative estimates, the former procedure was selected.

Problems associated with the finite-difference approximations

Nelder and Mead (1965a) discuss the problems of accurately locating the stationary point of the quadratic approximation. It is necessary to steer a course between two hazards. From one viewpoint the simplex can become so small that the estimated partial derivatives consist largely of roundoff errors incurred in calculating the responses that appear in the finite-difference formulae. On the other hand, the simplex can become so large that the quadratic approximation is poor, and the derivatives are correspondingly biased.

As noted by the authors, the former hazard will usually be the important one, and it may then be necessary to *enlarge* the final simplex before adding the extra points. They therefore suggest, in that case, to double the distance of each vertex V_i, $i = 1,2,\ldots,n+1$, from the centroid, $\bar{V}$, until the corresponding response value, F_i, exceeds that at the centroid, $F(\bar{V})$, by more than a given constant ϵ_3, that is, for the i-th vertex (in relative terms):

$$|[F_i - F(\bar{V})]/F(\bar{V})| > \epsilon_3 \qquad [A1.38]$$

The choice of this constant would depend on the rounding error attached to the evaluation of a "*typical*" response. According to Nelder and Mead, it would need to be *at least* 10^3 times that value if acceptable estimates of the derivatives were to be obtained allowing the stationary point to be located with the highest possible precision.

It is important to realize that the degree of "*exactness*" of a response is seen here as the error that occurs in *computing* that value due, among other things, to the *limitations of the machine*, and is by no means intended to include the accuracy to which the mathematical function reflects some process in the real world. An analysis of the sequence of calculations necessary to generate a response is therefore needed as a first step in estimating the constant ϵ_3. Hence, applying the principles of *propagation of uncertainties in functions* to equation A1.2 (Baird, 1962) leads to the following development.

Replacing for convenience $\hat{\sigma}_i^{-2}$ by w_i, equation A1.2 can be rewritten, for the k-th set of parameters, as:

$$\chi_k^2 = \Sigma_i w_i [Y_i - \hat{Y}_i^k]^2 = \Sigma_i c_i^k$$

$$i = 1,2,\ldots,m \qquad [A1.39]$$

Thus, taking the partial derivative of the above expression with respect to each term that carries an uncertainty yields, for the i-th term of the summation:

$$\partial c_i^k / \partial \hat{Y}_i^k = -2w_i[Y_i - \hat{Y}_i^k] \qquad [A1.40]$$

since w_i and Y_i are considered *exact* values.

The error in computing c_i^k is then given by:

$$e(c_i^k) = 2w_i[Y_i - \hat{Y}_i^k]\, e(\hat{Y}_i^k) \qquad [A1.41]$$

where $e(\hat{Y}_i^k)$ is the error incurred in evaluating the i-th predicted value of the dependent variable Y with the k-th set of parameters. This error is the *global truncation error* that arises when a value is obtained by numerical integration as a result of the *local truncation errors* from all the steps taken to obtain $\hat{Y}_i^k$. The estimation of that uncertainty is covered in details in Shampine and Gordon (1975) and will not be repeated here. Hence, the most "*pessimistic*" estimate of the error to be expected in calculating the response x_k^2 is given by:

$$\max e(x_k^2) = \Sigma_i \left| 2w_i[Y_i - \hat{Y}_i^k]\, e(\hat{Y}_i^k) \right|$$
$$i = 1,2,\ldots,m \qquad [A1.42]$$

The constant ϵ_3 is then defined, following Nelder and Mead's suggestion, as:

$$\epsilon_3^k = 1000\, [e(x_k^2)/F_k] \qquad [A1.43]$$

where the term $[e(x_k^2)/F_k]$ corresponds to the k-th *relative* *chi-square error*.

Since the quadratic optimization utilizes the responses at many vertices, our concern is not with an exact value of the "*accuracy*" at only one location. Rather, we are interested in obtaining a *good bound* on the error in the computed value for *any vertex*. A good estimate of the accuracy should therefore be obtained at a point which appears to be representative of the whole. The choice of the *centroid* of the final simplex as the "*typical*" vertex providing the k-th set of parameters needed in the evaluation of equation A1.42 seems consequently appropriate since, in the case of a too small simplex, this point will have a response value representing basically the "*average*" of all the other responses. This choice is further supported by the fact that the enlargement of the simplex is carried out, as suggested by Nelder and Mead, with respect to that latter point.

Failed enlargement

In the context of constrained optimization, provision must be made in the algorithm for the very rare occurrence of a *failed enlargement*. This could occur if the final simplex settles near a boundary and, upon expansion, one of the vertices is pushed beyond that limit. In that case, we propose, in a way similar to the procedure adopted for a *failed shrinkage*, to restore the faulty vertex to its previous valid position and to continue the expansion with the other points. This process is nevertheless of more consequences since, because of this failed expansion, there is no guarantee as to the unbiasedness of the optimized parameters. The final results must therefore be interpreted with care if they prove to be true optimum values.

Estimation of a_0

Finally, it remains to evaluate the constant scalar a_0. Thus, solving equation A1.24 at $\mathbf{x}_1$ yields the following expression:

$$F(\mathbf{x}_1) = a_0 + \mathbf{a}' \mathbf{x}_1 + .5\ \mathbf{x}_1' \mathbf{B}\ \mathbf{x}_1 \qquad [A1.44a]$$

But since by virtue of the new coordinate system, $\mathbf{x}_1$ is a null vector, the second and third term of the expression vanish and a_0 is consequently defined simply as the response value at the origin vertex, i.e.:

$$F(x_1) = a_0 = \overline{F_1} \qquad [A1.44b]$$

12.4 The role of the Hessian matrix

As discussed earlier, all numerical search methods seek local optima. Thus, once a particular solution has been found by an appropriate search technique, it is important to check that other, better points do not exist. In other words, it is desirable to examine the "*nature*" of the result. This examination will ensure, for example, that the solution is in fact optimal, and not a *saddle point* or a false stationary value, such as might be obtained by being stranded on a ridge. This study of the *stationary point* is, as we will see, based on the intrinsic characteristics of quadratic functions in the neighbourhood of an extremum. The role and importance of the Hessian matrix become then evident.

12.4.1 The Hessian matrix as part of optimality conditions

To examine an *unconstrained* optimum we use the rules of elementary calculus. Thus, as a result of the expected behavior of derivatives in the vicinity of an extremum, the following conditions are considered *sufficient* for **x*** to characterize a strong *local minimum* of F(**x**), as sought for in any least-squares optimization (details on the verification of these conditions can be found in Gill *et al.*, 1981):

C 1. **x*** is a stationary point of F(**x**), that is, the gradient vector $\nabla F(\mathbf{x})$ vanishes at **x***. Observe that this condition is implicitly fulfilled by use of equation A1.27.

C 2. The Hessian matrix of F(**x**) is *positive definite* at **x***, that is, the determinants of all the principal minors of $\nabla^2 F(\mathbf{x}^*)$ are *strictly* greater than zero. Note that this condition can be extended to $\mathbf{x}_i$, point where the elements of the Hessian matrix are currently evaluated, since, by *continuity*, $\nabla^2 F(\mathbf{x}^*)$ remains positive definite in some neighbourhood of **x***.

Although basically applicable only to the unconstrained case, these conditions are still valid for a *constrained* minimum if the stationary point occurs at a location **x*** which is in the *interior* of the feasible region. The constraints are in that case irrelevant as far as the *local nature* of the minimum is concerned (Bard, 1974). Hence, any point that complies with these conditions can be considered as a *true solution* and the search brought to a halt. On the other hand if the study of the Hessian matrix reveals that this is not a stationary value, we are then fully justified to pursue the search in quest of a better minimum. The Hessian matrix can consequently be seen as an "*information*" matrix from which not only the character of the stationary point can be studied but the behavior of F(x) in a neighbourhood of **x*** determined.

12.4.2 The Hessian matrix and the covariance matrix of the estimates

The search technique presently under study is a procedure which enables us to compute an estimate **P*** for the parameter vector **P** given the vector of experimental data **Y** (referring to the notation of equations A1.1 and A1.2). If the experiments which yielded our data were to be repeated, we would obtain different values of **Y** as can be expected from any stochastic processes. Application of the least-squares *estimator*, i.e., the chi-square statistic (equation A1.2), to the new data, would consequently yield different values of **P***. We see then that the estimates **P*** are themselves *random variables*, possessing a certain *probability distribution* which depends both on the nature of the estimator function and on the distribution of the observed random variable Y. This distribution, termed the *sampling distribution* of the chosen estimator can shed light on the reliability of the estimates since it represents the manner in which the estimates would vary in response to the random variations we expect to occur from one data sample to another. The feature of the sampling distribution that is of most interest to us is, therefore, its *covariance matrix*.

Accordingly, without going in the details of the derivation since it is far beyond the scope of this presentation, it can be shown that the covariance matrix of *least-squares estimates* with *normal distribution* is well approximated by the *inverse* of the Hessian matrix evaluated at the optimum (Bard, 1974). Hence, if we assume that the residuals, i.e., the difference between the observed Y and the predicted Y*, have normal equal-variance distribution, the *standard error* (SE) of any estimate is then given, for the i-th parameter estimate, by:

$$SE_i = \{[2F(x*)/(m-n)]C_{ii}\}^{1/2} \qquad i = 1,2,\ldots,n \qquad [A1.45]$$

where F(x*) is the response at the stationary point, m is the total number of observations, n the number of parameters fitted and C_{ii} the i-th element of the main diagonal of the Hessian matrix given, in terms of the *original coordinate system*, by:

$$C = Q\,B^{-1}\,Q' \qquad [A1.46]$$

where Q is the transformation matrix and B the Hessian matrix in terms of the *unit coordinate system*.

It appears therefore from the preceding discussion that the final quadratic fitting represents a powerful adjunct to the basic Simplex algorithm. Indeed, as presented, the procedure not only permits to accurately locate the stationary point but more importantly, particularly in least-squares minimization problems, allows to estimate a confidence region for the parameters from which sound conclusions can be drawn.

12.5 Practicalities

The final section of the present analysis will be devoted to discussing certain practical difficulties inherent in utilizing the search algorithm. We will cover important features such as the selection of good initial guess for the values of the parameters, scaling of the variables, the choice of proper convergence test coefficients and finally the assessment of the validity of the solution. We will conclude by presenting a flow chart of the algorithm as implemented for the problem under study.

12.5.1 The initial guess of the parameters

The optimization procedure that we have described requires that the user supply an *initial guess* for the values of the parameters. The starting simplex is then generated from these trial values and the search is initiated based on this initial information. The choice of a good initial guess represents therefore the difference betwen success and failure in locating the optimum, or between rapid and slow convergence to the solution since it directs to a certain extent the exploration of the response hyperspace. In the case of constrained optimization this is particularly crucial since these initial estimates *must lie inside* the feasible region for the search to proceed. Unfortunately, while the existing algorithm controls the path of the search, we must rely heavily on intuition and prior knowledge in selecting the initial guesses since sophisticated initialization techniques are often cumbersome and are simply not worth implementing. Bard (1974) suggests therefore that unless computer time is *exceptionally* scarce or expensive one attempt to estimate the parameters by "*brute force*". Thus, the most obvious method for selecting the initial guesses is to make the most of the available information such as estimates calculated from previous experiments, known values from similar systems or values computed from theoretical considerations. The user then modifies the initial guesses according to the results obtained after trial runs until proper values are determined.

Independently of the choice of good initial guess of the parameters, it remains to discuss the procedure adopted to set up the starting simplex. As a general rule we can state that the vertices of the initial simplex must not be too far from each other, especially in constrained optimization, if we want to be sure that all points will lie inside the feasible region. On the other hand, it is preferable to maintain a certain "*distance*" between the vertices if we want the simplex to react efficiently at the onset of the search. Fredenslund, Gmehling and Rasmussen (1977) therefore propose that a good compromise can be achieved by building the initial simplex by changing each of the initial parameters one at a time by 10%. Thus, for a case with three parameters for which the initial guess is P_1, P_2 and P_3, the vertices of the initial simplex would be (P_1, P_2, P_3), $(1.1P_1, P_2, P_3)$, $(P_1, 1.1P_2, P_3)$ and $(P_1, P_2, 1.1P_3)$.

12.5.2 Scaling of the parameter values

Scaling difficulties can occur when the value of one of the terms in the model function is of a much different order of magnitude than another relative to the significant figures in each term. The model function is then intrinsically insensitive to changes in the values of the parameters in the small term. Scaling problems can also occur when the parameters themselves are different in magnitudes due simply to the units in which they are expressed. The search is then performed in a space which is strongly "*distorted*" compromising the success of the operation. It becomes therefore necessary in both cases to modify the model function and/or to convert the variables in such a way that they display certain desirable properties during the optimization process.

The first basic rule of *scaling* is that the variables of the scaled problem should be of *similar magnitude* and, as much as possible of order unity in the region of interest, the aim being to cause each variable to be of similar "*weight*" during the optimization (Gill *et al.*, 1981). In the actual problem, this is ensured by performing a *dimensional analysis* of the model differential equations. This analysis basically consists in taking each term of the model functions together with appropriate and representative characteristics of the system under study and to combine them in suitable *dimensionless groups*. The resulting relations are then composed of terms of similar magnitude since all variables which are part of them are now independent of any unit system. Details on the performed dimensional analysis can be found elsewhere (see section 4.1.2).

The second basic rule is that the model should contain only *significant* parameters, that is, parameters that contribute *sensibly* in explaining the behavior of the experimental data. This prevents the search from being critically affected as it would have to proceed in an "*overdimensioned*" space. In the situation where the model is derived theoretically this does not usually present a problem since the parameters have physical significance but this can occur when *empirical models* are fitted. Special techniques can then be used to ensure that the model formulation will lead to a successful optimization (Gill *et al.*, 1981). Hence, the user should examine carefully the above possibilities when the optimization process behaves strangely, that is, for example, when numerous consecutive boundary violations are encountered, when the determinant of one of the principal minor of the Hessian matrix is systematically negative or when the standard error of an estimate is greater than the value of that estimate.

12.5.3 The choice of appropriate convergence test coefficients

The choice of proper values for the test coefficients ϵ_1 and ϵ_2 (equations A1.20 and A1.21) determines, as we have seen, the quality of the search achieved by the Simplex algorithm. We have also discuss the limitations of the latter approach and the need for a quadratic fitting to conclude the optimization. The question of selecting appropriate values for those coefficients is then equivalent to deciding which proportion of the search should be handled by the Simplex procedure versus the quadratic fitting. Hence, as a general rule we propose that most of the search be done by the former procedure since the performance of the quadratic approximation is greatly dependent on the fit being performed in the *neighboorhood* of the true optimum. The values of ϵ_1 and ϵ_2 must then be as small as possible though within certain limits based on the accuracy of the computations.

Indeed, a close look at equation A1.20 shows that ϵ_1 cannot take a value which is smaller than the relative chi-square error (equation A1.43) attached to the evaluation of any response. This is obvious since there is no meaning in forcing the coefficient of variation of the converged simplex, i.e., the *dispersion* of all the responses with respect to the mean, to be smaller than the inherent "*machine*" error related to the calculation of each of them.

The impact of such a limit is important since it determines the number of complete simplex searches allowed to find the optimum parameters. Indeed, as described previously, if the quadratic fitting does not lead to a true optimum another simplex search is initiated with tighter convergence coefficients. This search will be permitted nevertheless only if the test criterion (ϵ_1) is greater than this limit. Otherwise, the procedure is stopped and a message is issued warning the user that convergence cannot be obtained in the present conditions.

The constant ϵ_2 on the other hand, is not limited by such constraint and in fact should be assigned a rather small value since it is basically used to discriminate between local and global extremum. We therefore suggest for safety sake to maintain ϵ_2 less or equal to the value of coefficient ϵ_1.

12.5.4 Assessment of the validity of the solution

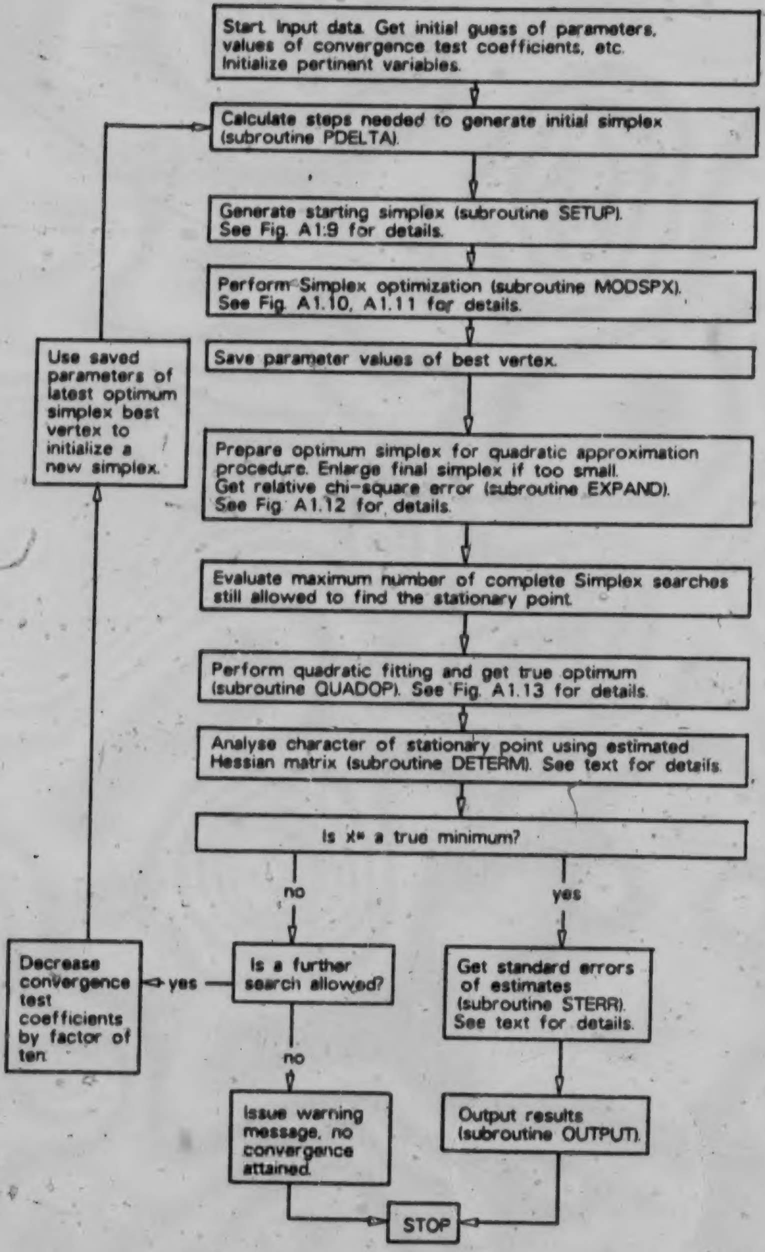
Referring to the present algorithm, the satisfaction of all the conditions for a successful termination means that a close approximation to a minimum has probably been found. However, the prudent user should apply some additional checks to ensure that the routine has not terminated prematurely.

Spendley (1969) therefore suggest that whenever a quadratic fit is performed and a sum of squares, i.e., a response is calculated at the indicated solution, one should compare this value with the *predicted response* as estimated by equation A1.29b. If true convergence has been achieved, that is, if the parameters are really the stationary estimates, the agreement should be satisfactory. Gill *et al.* (1981) states furthermore that a "*relatively small*" value of the response at the optimum should be an additional indication that an acceptable solution has been found. Futher confidence can finally be obtained by *restarting* the algorithm with a new set of initial parameters. However, it is important to choose a point that is not too close to the old initial point or any of the previous iterates. Preferably, the new initial estimate should be on the "*opposite side*" of the final value from the previous starting point.

12.6 Flow chart of the present algorithm

We will now summarize the preceding analysis by presenting a flow chart of the Simplex method and the quadratic approximation as implemented for the problem under study. The following diagrams describe only the *search procedure* and consequently do not provide details on the integration routines used to generate the predicted function values. This can be found elsewhere (see Appendix 2).

The present Simplex algorithm is based on the *PASCAL* code written by Cooper (1981). Substantial modifications have been made to the basic code to suit the quadratic procedure and to comply more closely with the features presented in the previous sections. We refer the reader to the complete listing of the program for an extensive description of the routines (see Appendix 2). Let us mention that other variations of the basic Simplex method have been proposed by several authors (Routh, Swartz and Denton, 1977; Ryan *et al.*, 1980; Nakai, 1981). They differ essentially from Nelder and Mead's, by the technique adopted to determine the location of the new vertex at each iteration. These versions appear particularly efficient in *interacting situations* such as in *computer-controlled instrumentation* where the algorithm encounters important random response fluctuations while optimizing the process variables. The interested reader may refer to the above references for more details.



Start. Input data. Get initial guess of parameters, values of convergence test coefficients, etc. Initialize pertinent variables.
Calculate steps needed to generate initial simplex (subroutine PDELTA).
Generate starting simplex (subroutine SETUP). See Fig. A1:9 for details.
Perform Simplex optimization (subroutine MODSPX). See Fig. A1.10, A1.11 for details.
Save parameter values of best vertex.
Use saved parameters of latest optimum simplex best vertex to initialize a new simplex.
Prepare optimum simplex for quadratic approximation procedure. Enlarge final simplex if too small. Get relative chi-square error (subroutine EXPAND). See Fig. A1.12 for details.
Evaluate maximum number of complete Simplex searches still allowed to find the stationary point.
Perform quadratic fitting and get true optimum (subroutine QUADOP). See Fig. A1.13 for details.
Analyse character of stationary point using estimated Hessian matrix (subroutine DETERM). See text for details.
Is X* a true minimum?
no
yes
Decrease convergence test coefficients by factor of ten.
yes
Is a further search allowed?
Get standard errors of estimates (subroutine STERR). See text for details.
no
Issue warning message, no convergence attained.
Output results (subroutine OUTPUT).
STOP

Figure A1.8 Flow chart of the optimization program (MAIN routine, program MCPERM).

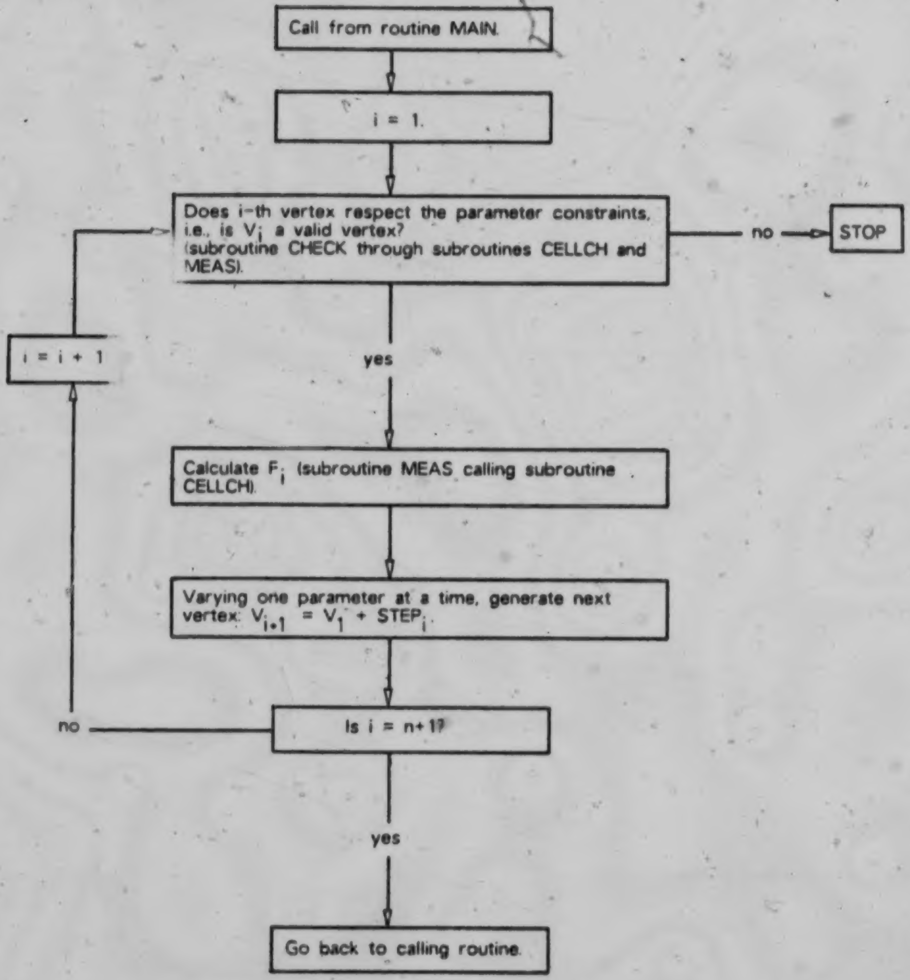

Call from routine MAIN.
i = 1.
Does i-th vertex respect the parameter constraints, i.e., is V_i a valid vertex? (subroutine CHECK through subroutines CELLCH and MEAS).
no
STOP
yes
i = i + 1
Calculate F_i (subroutine MEAS calling subroutine CELLCH).
Varying one parameter at a time, generate next vertex: V_i+1 = V_i + STEP_i.
no
Is i = n+1?
yes
Go back to calling routine.

Figure A1.9 Details of initialization procedure (subroutine SETUP).

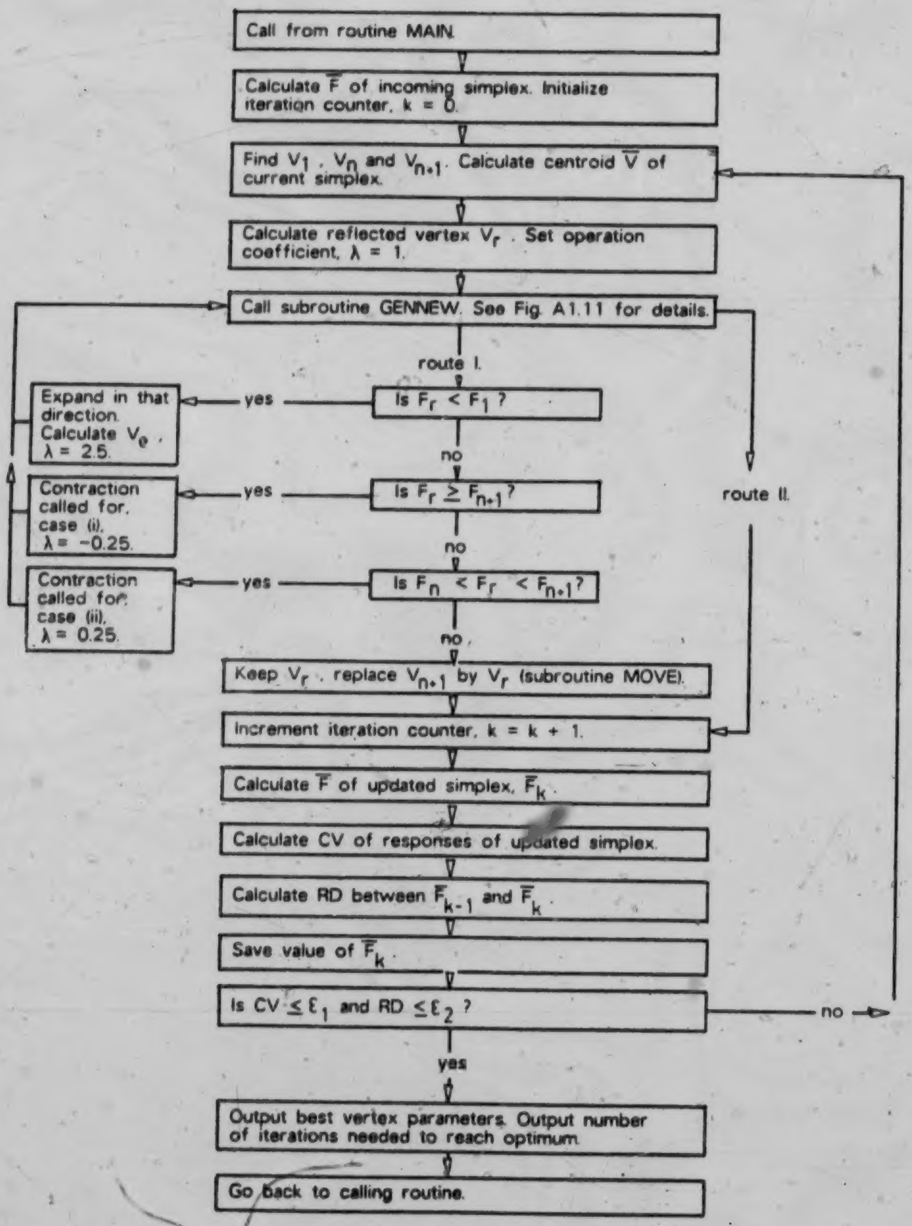

Call from routine MAIN.
Calculate $\overline{F}$ of incoming simplex. Initialize iteration counter, $k = 0$.
Find V_1, V_n and V_{n+1}. Calculate centroid $\overline{V}$ of current simplex.
Calculate reflected vertex V_r. Set operation coefficient, $\lambda = 1$.
Call subroutine GENNEW. See Fig. A1.11 for details.
route I.
route II.
Is $F_r < F_1$?
yes
Expand in that direction. Calculate V_e, $\lambda = 2.5$.
no
Is $F_r \geq F_{n+1}$?
yes
Contraction called for, case (i), $\lambda = -0.25$.
no
Is $F_n < F_r < F_{n+1}$?
yes
Contraction called for, case (ii), $\lambda = 0.25$.
no
Keep V_r, replace V_{n+1} by V_r (subroutine MOVE).
Increment iteration counter, $k = k + 1$.
Calculate $\overline{F}$ of updated simplex, $\overline{F}_k$.
Calculate CV of responses of updated simplex.
Calculate RD between $\overline{F}_{k-1}$ and $\overline{F}_k$.
Save value of $\overline{F}_k$.
Is CV $\leq \varepsilon_1$ and RD $\leq \varepsilon_2$?
no
yes
Output best vertex parameters. Output number of iterations needed to reach optimum.
Go back to calling routine.

Figure A1.10 Details of Simplex optimization procedure (subroutine MODSPX).

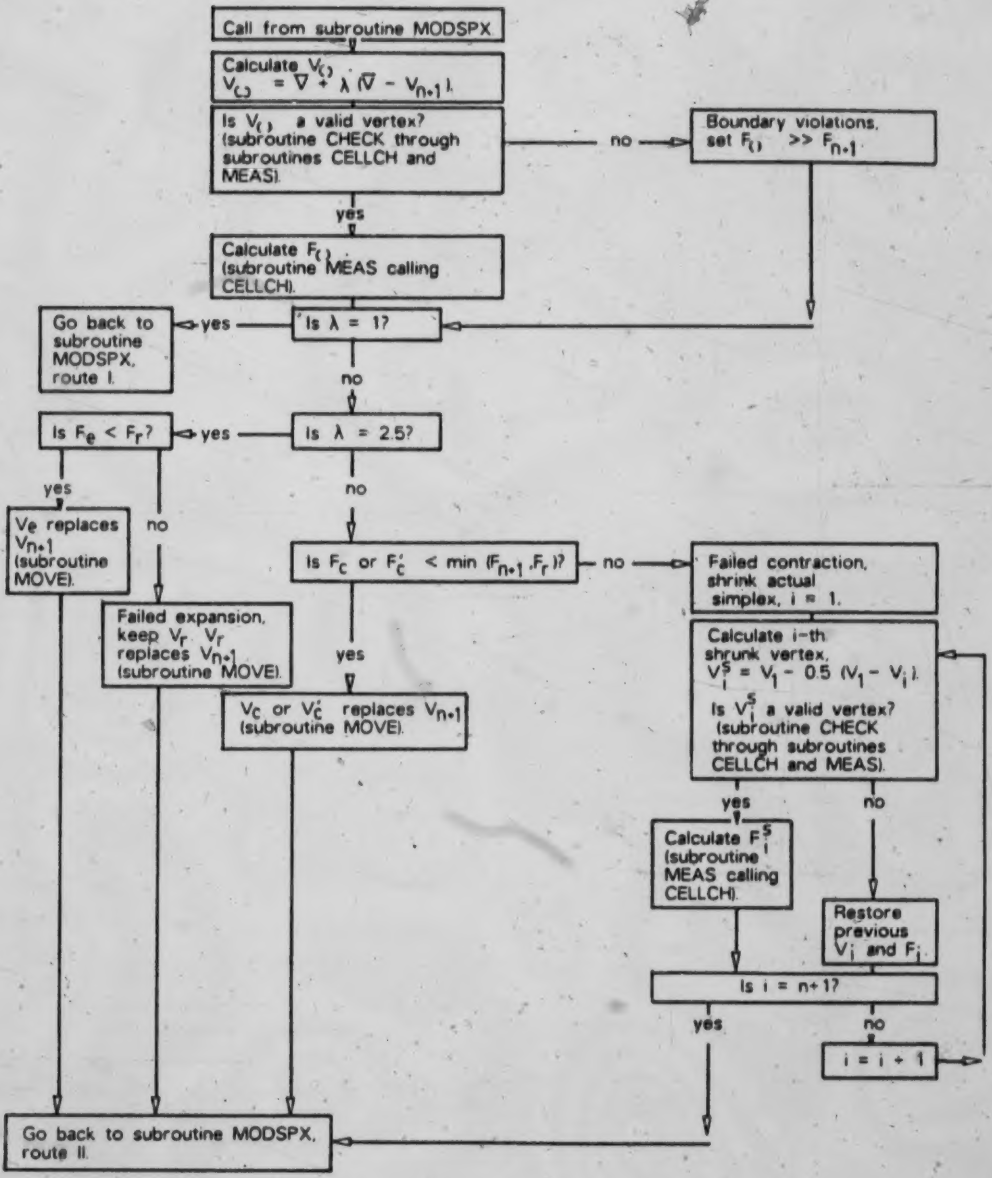

Call from subroutine MODSPX
Calculate $V_{( )}$
$V_{( )} = \bar{V} + \lambda(\bar{V} - V_{n+1})$.
Is $V_{( )}$ a valid vertex? (subroutine CHECK through subroutines CELLCH and MEAS).
no
Boundary violations, set $F_{( )} >> F_{n+1}$
yes
Calculate $F_{( )}$. (subroutine MEAS calling CELLCH).
Go back to subroutine MODSPX, route I.
yes
Is $\lambda = 1$?
no
Is $F_e < F_r$?
yes
Is $\lambda = 2.5$?
yes
no
Ve replaces V_{n+1} (subroutine MOVE).
no
Is F_c or $F_c' < \min(F_{n+1}, F_r)$?
no
Failed contraction, shrink actual simplex, i = 1.
Failed expansion, keep V_r. V_r replaces V_{n+1} (subroutine MOVE).
yes
V_c or V_c' replaces V_{n+1} (subroutine MOVE).
Calculate i-th shrunk vertex, $V_i^S = V_1 - 0.5\,(V_1 - V_i)$.
Is V_i^S a valid vertex? (subroutine CHECK through subroutines CELLCH and MEAS).
yes
no
Calculate F_i^S (subroutine MEAS calling CELLCH).
Restore previous V_i and F_i.
Is i = n+1?
yes
no
i = i + 1
Go back to subroutine MODSPX, route II.

Figure A1.11 Details of Simplex optimization procedure (subroutine GENNEW).

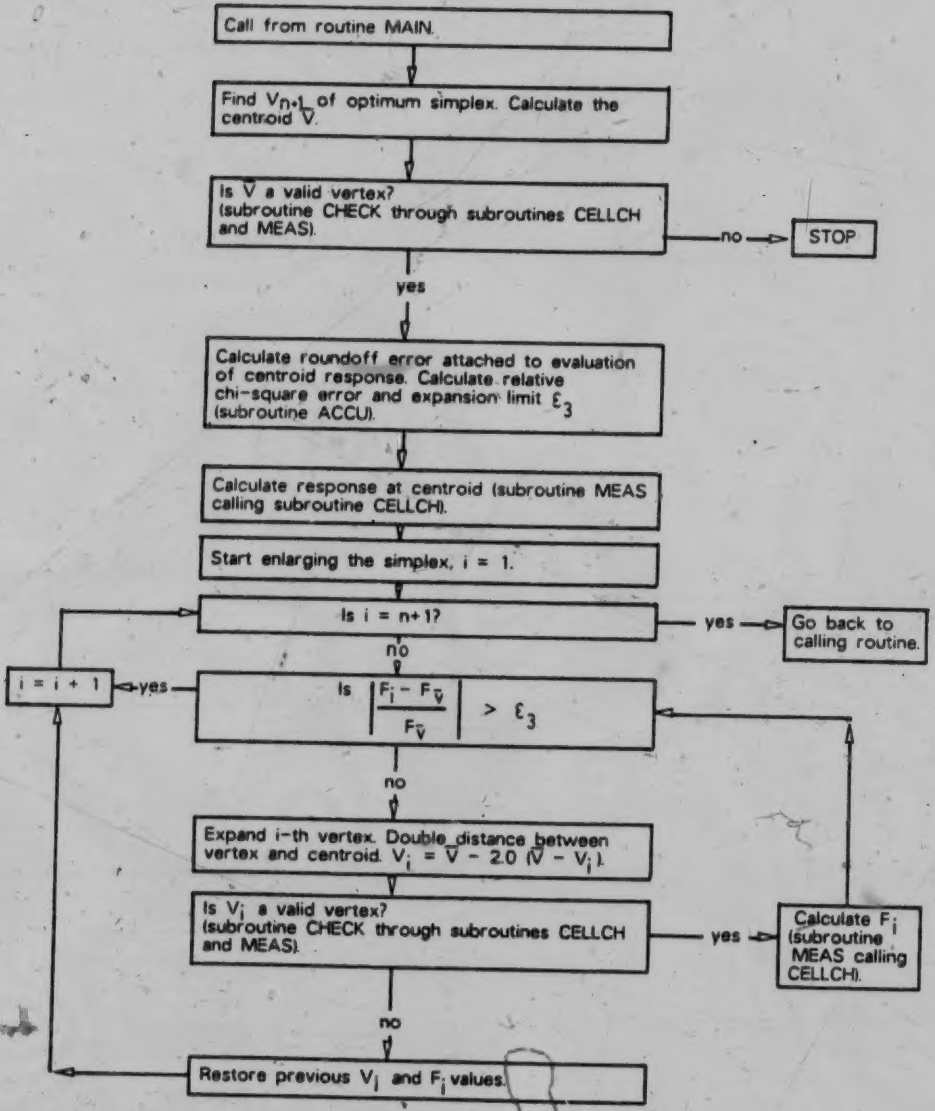

Call from routine MAIN.
Find V_{n+1} of optimum simplex. Calculate the centroid $\bar{V}$.
Is $\bar{V}$ a valid vertex? (subroutine CHECK through subroutines CELLCH and MEAS).
no
STOP
yes
Calculate roundoff error attached to evaluation of centroid response. Calculate relative chi-square error and expansion limit ε_3 (subroutine ACCU).
Calculate response at centroid (subroutine MEAS calling subroutine CELLCH).
Start enlarging the simplex, i = 1.
Is i = n+1?
yes
Go back to calling routine.
no
i = i + 1
yes
Is $\left|\frac{F_i - F_{\bar{V}}}{F_{\bar{V}}}\right| > \varepsilon_3$
no
Expand i-th vertex. Double distance between vertex and centroid. $V_i = \bar{V} - 2.0\,(\bar{V} - V_i)$.
Is V_i a valid vertex? (subroutine CHECK through subroutines CELLCH and MEAS).
yes
Calculate F_i (subroutine MEAS calling CELLCH).
no
Restore previous V_i and F_i values.

Figure A1.12 Details of enlargement procedure (subroutine EXPAND).

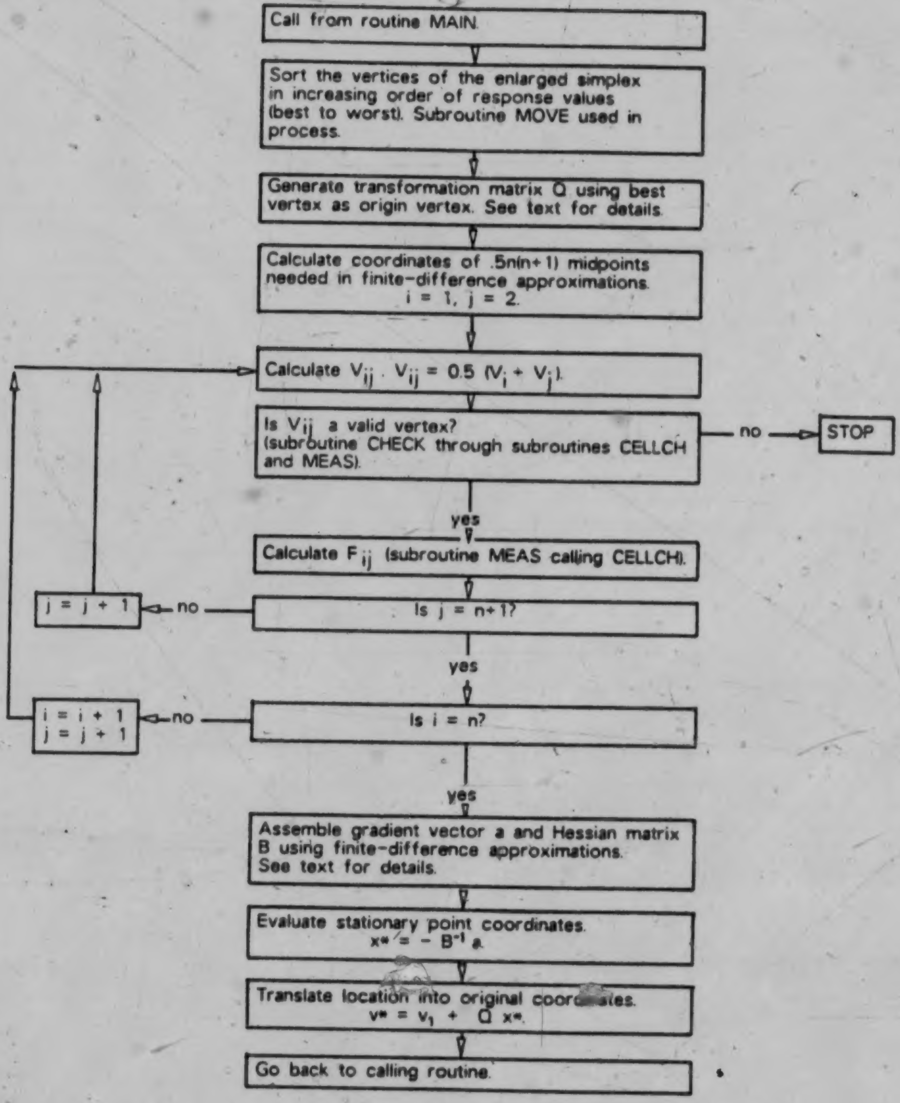

Call from routine MAIN.
Sort the vertices of the enlarged simplex in increasing order of response values (best to worst). Subroutine MOVE used in process.
Generate transformation matrix Q using best vertex as origin vertex. See text for details.
Calculate coordinates of .5n(n+1) midpoints needed in finite-difference approximations. i = 1, j = 2.
Calculate V_ij . V_ij = 0.5 (V_i + V_j).
Is V_ij a valid vertex? (subroutine CHECK through subroutines CELLCH and MEAS).
no
STOP
yes
Calculate F_ij (subroutine MEAS calling CELLCH).
j = j + 1
no
Is j = n+1?
yes
i = i + 1
j = j + 1
no
Is i = n?
yes
Assemble gradient vector a and Hessian matrix B using finite-difference approximations. See text for details.
Evaluate stationary point coordinates. x* = - B^-1 a.
Translate location into original coordinates. v* = v_1 + Q x*.
Go back to calling routine.

Figure A1.13 Details of the quadratic approximation procedure (subroutine QUADOP).

13. APPENDIX 2: LISTING OF PROGRAM *MCPERM*

13.1 Introduction

In the following pages the reader will find the complete listing of the program used to carry out the numerical estimation of membrane macroscopic permeability coefficients from cell volume change data (see Chapter 5). Examples of data and output files are also provided. The codes have been written in standard *FORTRAN IV* (Cress, Dirksen and Graham, 1970) and should therefore be compatible with most *FORTRAN* compilers. Since the codes include extensive comments, the reader is referred to the listing for the complete description of the algorithm.

13.2 General description of the program

The program is divided into 4 units: a "main" driver, a set of subroutines referred to as "cell behavior" routines, a set of subroutines referred to as "optimization" routines and, finally, a group of subroutines referred to as "utility" routines.

13.2.1 The main driver

This unit controls the overall progress of the numerical estimation of the permeability coefficients. It calls sequentially all the routines necessary to perform the optimization search. A detailed flow chart of the main driver is given in Appendix 1 (Figure A1.8).

```
C
C     PROGRAM NAME   -MCPERM
C
C     LATEST VERSION  -APRIL 2, 1985.
C
C     WRITTEN BY  -CHRISTIAN TOUPIN
C                  FOOD ENGINEERING GROUP
C                  DEPARTMENT OF FOOD SCIENCE
C                  UNIVERSITY OF ALBERTA
C                  EDMONTON,ALBERTA.
C
C     PURPOSE  -THIS PROGRAM USES A MODIFIED SIMPLEX METHOD
C               TO FIT A SYSTEM OF DIFFERENTIAL EQUATIONS
C               (DESCRIBING THE TRANSPORT OF MATERIAL ACROSS
C               CELL MEMBRANES) TO A GIVEN SET OF EXPERIMENTAL
C               DATA IN ORDER TO ESTIMATE THE EQUATION PARAMETERS
C               (MACROSCOPIC PERMEABILITY COEFFICIENTS).
C
C     REMARKS  -THE TREATMENT IS LIMITED TO THE ANALYSIS OF THE
C               PASSIVE MASS TRANSFER OF NON-ELECTROLYTES ACROSS
C               CELL MEMBRANES.
C
C               THE PROGRAM HAS BEEN DESIGNED TO ALLOW FOR THE
C               ANALYSIS OF DATA CORRESPONDING TO RUNS MADE AT
C               DIFFERENT TEMPERATURES AND/OR WITH DIFFERENT
C               CONCENTRATIONS OF THE SAME SOLUTE(S) IN A GIVEN
C               OSMOTICUM (BATHING SOLUTION).
C
C     PROGRAMMING NOTES
C
C                     1.   UTILITY ROUTINES ARE USED IN SOME SUBR.
C                          (QUADOP, DETERM, STERR AND CELLCH)
C                          GREAT CARE SHOULD BE TAKEN TO MAKE SURE
C                          THAT THE PARAMETERS NEEDED BY THESE SUBR.
C                          ARE STILL VALID IF CHANGES ARE MADE TO
C                          THE PROGRAM.
C
C                          IMSL I/O CONTROL ROUTINE (USETIO) AND A
C                          PLOTTING ROUTINE (USPLTD) ARE UTILIZED
C                          IN SUBROUTINE OUTPUT TO PLOT EXPERIMENTAL
C                          AND PREDICTED DATA. CONSULT IMSL LIBRARY
C                          FOR DETAILS ON THEIR USE.
C
C                     2.   ALL CALCULATIONS ARE DONE IN DOUBLE-
C                          PRECISION TO ENSURE MINIMUM ROUNDING
C                          ERRORS (SEE SUBR. ACCU). IF ANY
C                          MODIFICATIONS ARE TO BE MADE TO THE
C                          ROUTINES, MAKE SURE TO USE FORTRAN
C                          DOUBLE-PRECISION BUILT-IN FUNCTIONS.
C
C                     3    THE USER MUST RESPECT THE FOLLOWING
C                          CONVENTIONS AS TO THE SIZE OF THE ARRAYS
C                          UTILIZED IN THE PROGRAM IF ANY CHANGES ARE
C                          TO BE MADE (SEE NOTE 1).
C
C                          THE DIMENSIONS OF THE ARRAYS ARE BASED ON
C                          A MAXIMUM NUMBER OF SOLUTES EXPECTED TO BE
C                          PRESENT IN ANY SYSTEM (SMAX). FROM THAT
C                          VALUE THE MAXIMUM NUMBER OF PARAMETERS THAT
C                          COULD HAVE TO BE OPTIMIZED IF ALL THESE
C                          SOLUTES WERE PERMEABLE CAN BE CALCULATED
C                          AS FOLLOWS:
C
C                                  PMAX = (SMAX+1) * (SMAX+2)
C
C                          FROM THESE 2 VALUES, 4 TYPES OF ARRAY SIZES
C                          CAN BE DEFINED:
C
C                         A. TYPE 1(T1) -DIMENSION IS DIRECTLY SMAX,
C                                        I.E., T1=SMAX.
C
C                         B. TYPE 2(T2) -DIMENSION IS RELATED TO PMAX
C                                        AS FOLLOWS: T2=PMAX+2.
C
C                         C. TYPE 3(T3) -DIMENSION IS RELATED TO PMAX
C                                        AS FOLLOWS:
C                                        T3=.5*(PMAX+1)*(PMAX+2)+PMAX.
C
C                         D. TYPE 4(T4) -DIMENSION IS RELATED TO THE
C                                        MAX. NUMBER OF EXPERIMENTAL
C                                        DATA EXPECTED TO BE READ BY
C                                        THE PROGRAM.
C                                        MINIMUM VALUE: PMAX+3.
C
C                          THE FOLLOWING TYPES ARE BASED ON THE DESIGN
C                          OF THE EXPERIMENTS.
C
C                         E. TYPE 5(T5) -DIMENSION IS RELATED TO MAX.
C                                        NUMBER OF TEMPERATURE TO BE
C                                        EXPECTED IN ANY ANALYSIS.
C
C                         F. TYPE 6(T6) -DIMENSION IS RELATED TO MAX.
C                                        NUMBER OF CONCENTRATION
C                                        LEVELS TO BE EXPECTED IN ANY
C                                        ANALYSIS.
C
C                         G. TYPE 7(T7) -DIMENSION IS RELATED TO THE
C                                        MAXIMUM NUMBER OF CASES TO BE
C                                        EXPECTED IN ANYONE OF THE
C                                        DATA SETS.
C
C                          TO ALTER ARRAY DIMENSIONS, THE USER SHOULD
C                          CONSULT THE COMMENTED DIMENSION STATEMENTS
C                          WHICH PRECEDE THE FORMAL ONES. IN ITS
C                          ACTUAL FORM A MAXIMUM OF 2 SOLUTES CAN BE
C                          HANDLED BY THE PROGRAM, THEREFORE SMAX=2,
C                          PMAX=12. CONSEQUENTLY T1=2, T2=14 AND
C                          T3=103. UP TO 4000 CASES CAN BE READ,
C                          THEREFORE T4=4000. A MAXIMUM OF 6
C                          TEMPERATURE AND 20 CONCENTRATION LEVELS
C                          ARE ALLOWED, THEREFORE T5=6 AND T6=20.
C                          FINALLY A MAXIMUM OF 120 CASES PER DATA
C                          SET IS ALLOWED, THEREFORE T7=120.
C
C                          NOTE THAT A "CONCENTRATION LEVEL" REFERS
C                          EITHER TO AN OSMOTICUM COMPOSITION
C                          OR TO A REPLICATE OF A GIVEN OSMOTICUM.
C
C                          THE USER SHOULD ALSO MODIFY ACCORDINGLY
C                          THE DIGIT VECTORS USED IN EXECUTION-TIME
```

```
C                            FORMATTING (SEE SUBROUTINES STERR AND
C                            CELLCH, INTEGER VARIABLE DECLARATIONS).
C
C                      4.    /COPT*/ COMMONS ARE RELATED TO THE
C                            "OPTIMIZATION" ROUTINES WHEREAS /CCELL*/
C                            COMMONS ARE RELATED TO THE "CELL BEHAVIOUR"
C                            ROUTINES.
C
C     VARIABLES
C
C        1. RELATED TO THE "OPTIMIZATION" ROUTINES.
C
C                        AM -MATRIX OF PARAMETER VALUES (COORDINATES)
C                            VS VERTICES.
C                    VV,WTV -VECTORS OF EXPERIMENTAL DEPENDENT VARIABLE
C                            VALUES (RELATIVE CELL VOLUMES) AND THEIR
C                            ASSOCIATED WEIGHTS, I.E., THE INVERSE OF
C                            THEIR ESTIMATED VARIANCES.
C                        XV -VECTOR OF INDEPENDENT VARIABLE VALUE
C                            (TIME), IN SEC.
C                     HOLDV -VECTOR USED TO SAVE THE OPTIMIZED PARAMETER
C                            VAL. BEFORE CALLING SUBR. QUADOP.
C                    DELTAV -VECTOR OF THE STEPS NEEDED TO SET UP THE
C                            FIRST SIMPLEX. GENERATED IN SUBR. PDELTA.
C                            USED IN SUBR. SETUP.
C                     RESPV -VECTOR OF THE CHI-SQUARE VAL. CORRESPONDING
C                            TO EACH VERTEX OF AM (COLUMNS OF AM).
C                    STERRV -VECTOR OF THE STANDARD ERRORS OF THE FINAL
C                            OPTIMIZED PARAMETERS.
C              VMAX,VMAXP1 -VMAX IS THE NUMBER OF PARAMETERS TO BE
C                            OPTIMIZED. VMAXP1 = VMAX + 1.
C     BEST,WORST,NEWPNT,   -POSITION INDEXES. BEST, WORST AND NEWPNT
C     TOTCNT                 GENERATED IN MODSPX (SEE SUBR. FOR DFN),
C                            TOTCNT IS THE TOTAL NUMBER OF COLUMNS IN
C                            AM ONCE THE MIDPOINTS HAVE BEEN GENERATED
C                            (SEE SUBR. QUADOP FOR DETAILS).
C                    TESTSF -FLAG TO INDICATE IF LISTING OF THE TEST
C                            VAR. AVERT AND SPXT (USED IN MODSPX) IS
C                            DESIRED (TESTSF=1 IF LISTING DESIRED).
C                    CHECKF -FLAG TO INDICATE IF THE PARAMETERS TO BE
C                            USED IN THE CALCULATION OF THE PREDICTED
C                            RESPONSES ARE VALID. GENERATED IN SUBR.
C                            CHECK (CHECK=1 IF NOT VALID).
C                     DETRF -FLAG USED IN SUBR. DETERM TO INDICATE IF
C                            BETAM MATRIX IS POSITIVE-DEFINITE.
C                            (DETRF=1 IF NOT) (SEE SUBR. DETERM).
C                    IERROR -ERROR FLAG TO SIGNAL MALFUNCTION OF
C                            UTILITY ROUTINES USED IN SOME SUBR.
C                            IN THAT CASE IERROR IS SET TO 1.
C                      ITER -ITERATION COUNTER. IT SHOWS THE NUMBER OF
C                            COMPLETE SIMPLEX SEARCHES NEEDED TO REACH
C                            A TRUE MINIMUM.
C             TOLSPX,TOLAVE -TOLERANCES BELOW WHICH SPXT AND AVERT MUST
C                            FALL. USED AS CRITERIA FOR STOPPING AN
C                            OPTIMUM SEARCH PROCESS (SEE SUBR. MODSPX).
C                    ITEMAX -MAXIMUM NUMBER OF ITERATIONS ALLOWED TO
C                            FIND AN OPTIMUM IN A GIVEN SIMPLEX SEARCH.
C                     ITEFL -FLAG TO INDICATE IF THE MAXIMUM NUMBER OF
C                            ITERATIONS ALLOWED IN ANY SIMPLEX SEARCH
C                            HAS BEEN REACHED. IN THAT CASE ITEFL IS SET
C                            TO 1.
C                    RCSERR -RELATIVE CHI-SQUARE ERROR VALUE USED IN
C                            ESTIMATING SPXMAX. THIS IS A MEASURE OF THE
C                            ACCURACY TO BE EXPECTED, ON AVERAGE, ON ANY
C                            CHI-SQUARE VALUE CALCULATED IN THE PROGRAM.
C                    SPXMAX -MAXIMUM NUMBER OF COMPLETE SIMPLEX SEARCHES
C                            STILL ALLOWED TO CONVERGE ON THE SOLUTION.
C                            THIS IS BASED ON THE RELATIVE PRECISION
C                            THAT CAN BE OBTAINED IN EVALUATING THE
C                            PREDICTED Y VALUES (SEE SUBR. ACCU).
C                    DLOG10 -FORTRAN DOUBLE-PRECISION BUILT-IN FUNCTION.
C                     IDINT -FORTRAN INTEGER BUILT-IN FUNCTION.
C                     PCERR -PERCENTAGE ERROR OF FINAL OPTIMIZED
C                            PARAMETERS.
C
C        2. RELATED TO THE "CELL BEHAVIOUR" ROUTINES.
C
C                            SEE SUBROUTINE INPUT FOR DEFINITIONS OF
C                            THE FOLLOWING VARIABLES.
C
C                            R, TEMPL, TEMPV, CONCL, SOLNUM, IMPNUM,
C                            PMVV, CSSOLM, CAREAO, VCO, RNOV, CSIOV,
C                            CASEM, TOLABS AND TOLREL.
C
C                       ADF -FLAG TO INDICATE POSSIBLE MALFUNCTION OF
C                            SUBROUTINE ODE IN SUBR. CELLCH. IN THAT
C                            CASE ADF=1.
C
C
C
C*****************************************************************
C                            MAIN PROGRAM
C*****************************************************************
C
C
C     DIMENSION AM(T2,T3),RESPV(T3),DELTAV(T2),HOLDV(T2),STERRV(T2).
C
        DIMENSION AM(14,103),RESPV(103),DELTAV(14),HOLDV(16),
     *            STERRV(14)
C
        DOUBLE PRECISION AM,RESPV,TOLSPX,TOLAVE,DELTAV,HOLDV,RCSERR,
     *                   DLOG10,STERRV,PCERR
C
        INTEGER VMAX,VMAXP1,WORST,NEWPNT,TOTCNT,TESTSF,CHECKF,DETRF,
     *          ITER,SPXMAX,IDINT,BEST,IERROR,SOLNUM,SOLNP1,ADF,
     *          IMPNUM,CONCL,TEMPL,ITEMAX,ITEFL
C
        COMMON /COPT1/ AM,RESPV
        COMMON /COPT2/ VMAX,VMAXP1,SOLNUM,SOLNP1,IMPNUM,CONCL,TEMPL
        COMMON /COPT4/ BEST,WORST,NEWPNT,TOTCNT
        COMMON /COPT5/ CHECKF,ADF
        COMMON /COPT6/ TOLSPX,TOLAVE,TESTSF,ITEMAX
C
C
C     INPUT DATA.
C
        CALL INPUT
C
C     INITIALIZE COUNTERS AND FLAGS.
C
```

```
      ITER=0
      TOTCNT=0
      CHECKF=0
      DETRF=0
100   CONTINUE
C
C  CALCULATE THE STEPS NEEDED TO GENERATE THE INITIAL SIMPLEX.
C
      CALL PDELTA(DELTAV)
C
C  MAKE N+1 MEASUREMENTS TO SET UP THE AM(PARAMETERS,VERTICES) MAT.
C
      CALL SETUP(DELTAV)
C
C  CHECK IF CHECKF HAS BEEN SET TO 1, I.E, THE PARAMETERS GENERATED
C  IN SETUP ARE NOT VALID, AND/OR IF ADF=1, I.E., MALFUNCTION OF
C  SUBR. ODE (IN SUBR. CELLCH).
C
      IF ((CHECKF .NE. 1) .AND. (ADF .NE. 1)) GO TO 40
      IF (ADF .EQ. 1) GO TO 666
      WRITE(2,2240)
2240  FORMAT(//,' ***** PARAMETERS NOT VALID IN SETUP. *****')
      GO TO 666
40    CONTINUE
C
C  NOW PERFORM SIMPLEX OPTIMIZATION. CALL THE SIMPLEX PROCEDURE.
C
      ITEFL=0
      CALL MODSPX(TOLSPX,TOLAVE,TESTSF,ITEMAX,ITEFL)
      IF (ITEFL .EQ. 0) GO TO 42
      WRITE(2,2242)
2242  FORMAT(//,' ***********  WARNING  ***********',/,
     @          ' *  MAXIMUM NUMBER OF ITERATIONS *',/,
     @          ' *  ALLOWED HAS BEEN REACHED.     *',/,
     @          ' *                                *',/,
     @          ' *  PROCEDURE ABORTED. NO OUTPUT *',/,
     @          ' *  PROVIDED SINCE IT MIGHT NOT   *',/,
     @          ' *  BE VALID.                     *',/,
     @          ' **********************************')
      GO TO 666
42    CONTINUE
C
C  CHECK FOR MALFUNCTION OF SUBR. ODE (IN SUBR. CELLCH). IF SO, STOP
C  THE WHOLE PROGRAM.
C
      IF (ADF .EQ. 1) GO TO 666
C
C  ONCE THE TRUE OPTIMUM IS REACHED, EXPAND THE FINAL SIMPLEX
C  AROUND THE CENTROID TO DECREASE THE POSSIBILITY THAT BETAM MAT
C  (TO BE CALCULATED IN QUADOP) CONSISTS LARGELY OF ROUNDOFF ERRORS
C  INCURRED IN CALCULATING THE RESPV VALUES (CHI-SQUARE VALUES)
C  SAVE THE VALUES OF THE OPTIMIZED PARAMETERS IN CASE OF FAILURE OF
C  THE QUADRATIC APPROXIMATION.
C
      DO 50 I=1,VMAX
50    HOLDV(I)=AM(I,BEST)
C
C  PERFORM THE EXPANSION USING EXPLIM VALUE AS LIMIT. THIS LIMIT
C  IS EVALUATED INTERNALLY (SEE SUBROUTINE ACCU).
C
      CALL EXPAND(RCSERR)
C
C  CHECK IF CHECKF AND/OR ADF HAVE BEEN SET TO 1. IF SO, STOP THE
C  WHOLE PROGRAM.
C
      IF ((CHECKF .NE. 1) .AND. (ADF .NE. 1)) GO TO 70
      IF (ADF .EQ. 1) GO TO 666
      WRITE(2,2260)
2260  FORMAT(//,' ***** CENTROID NOT VALID IN EXPAND. *****')
      GO TO 666
70    CONTINUE
      SPXMAX=IDINT(DLOG10(TOLSPX/RCSERR)+0.25D0)
      WRITE(2,2250) SPXMAX
2250  FORMAT(/,' MAXIMUM NUMBER OF SIMPLEX SEARCHES STILL ALLOWED ',
     @       I4)
C
C  PERFORM THE QUADRATIC FITTING AND GET THE TRUE OPTIMUM.
C
      CALL QUADOP(IERROR)
      IF (IERROR .EQ. 1) GO TO 666
C
C  CHECK IF CHECKF AND/OR ADF HAVE BEEN SET TO 1. IF SO, STOP THE
C  WHOLE PROGRAM.
C
      IF ((CHECKF .NE. 1) .AND. (ADF .NE. 1)) GO TO 80
      IF (ADF .EQ. 1) GO TO 666
      WRITE(2,2270)
2270  FORMAT(//,' ***** GENERATED HALFPOINT NOT VALID. *****')
      GO TO 666
80    CONTINUE
C
C  CHECK IF WE HAVE A TRUE MINIMUM AT THE STATIONARY POINT.
C
      CALL DETERM(DETRF,IERROR)
      IF (IERROR .EQ. 1) GO TO 666
C
C  REPEAT THE SIMPLEX SEARCH PROCESS STARTING WITH THE SAVED
C  OPTIMIZED PARAMETERS (HOLDV) AS INITIAL GUESS IF THE OPTIMUM
C  IS NOT A MINIMUM, I.E. DETRF=1, OR UNTIL SPXMAX .LE. 0, I.E.,
C  THERE IS NO MORE SIMPLEX SEARCH ALLOWED TO FIND THE OPTIMUM.
C  THE TOLERANCES TOLSPX AND TOLAVE ARE DECREASED BY A FACTOR OF 10.
C
      ITER=ITER+1
      TOLSPX=TOLSPX/10.0D0
      TOLAVE=TOLAVE/10.0D0
      DO 90 I=1,VMAX
90    AM(I,1)=HOLDV(I)
      IF (.NOT.((DETRF .EQ. 0) .OR. (SPXMAX .LE. 0))) GO TO 100
      IF ((DETRF .NE. 0) .AND. (SPXMAX .LE. 0)) GO TO 120
C
C  IF A TRUE OPTIMUM HAS BEEN FOUND, GET THE STANDARD ERRORS OF
C  THE OPTIMIZED PARAMETERS.
C
      CALL STERR(STERRV,IERROR)
      IF (IERROR .EQ. 1) GO TO 666
C
C  OUTPUT THE FINAL OPTIMUM VALUES AND THEIR ERRORS.
C
      WRITE(2,2280)
2280  FORMAT(//,17X,'PARAMETERS',8X,'STANDARD ERRORS',8X,
     &        'PERCENT ERRORS',/)
      DO 150 I=1,VMAX
      PCERR=(STERRV(I)/AM(I,TOTCNT+1))*100.0D0
      WRITE(2,2290) I, AM(I,TOTCNT+1), STERRV(I), PCERR
2290  FORMAT(7X,'P(',I2,')',4X,1PD11.4,11X,D11.4,12X,0PF6.2)
150   CONTINUE
      WRITE(2,2300) RESPY(TOTCNT+1)
2300  FORMAT(/,' CHI-SQUARE VALUE AT THE OPTIMUM: ',1PD10.3)
      WRITE(2,2310) ITER
2310  FORMAT(/,' NUMBER OF COMPLETE SIMPLEX SEARCHES: ',I4)
C
C  OUTPUT TABLE OF PREDICTED VS OBSERVED Y AT OPTIMUM AND THE
C  DIFFERENCES BETWEEN THESE VALUES.
C
      CALL OUTPUT
      GO TO 666
120   CONTINUE
      WRITE(2,2320)
2320  FORMAT(///,' ************  WARNING  ************',/,
     &              ' *  THE PROCEDURE DID NOT CONVERGE *',/,
     &              ' *  ON A TRUE MINIMUM.             *',/,
     &              ' *                                 *',/,
     &              ' *  PROCEDURE ABORTED. NO OUTPUT   *',/,
     &              ' *  PROVIDED SINCE IT MIGHT NOT    *',/,
     &              ' *  BE VALID.                      *',/,
     &              ' ***********************************')
666   CONTINUE
      STOP
      END
```

13.2.2 The "cell behavior" routines

This unit contains all the routines which are necessary to simulate the "theoretical" behavior of a hypothetical cell exposed to an osmotic solution (see section 4.1).

Subroutines INPUT and OUTPUT are called, respectively, to input the pertinent data for the optimization run and to output the results of the search. Note that the latter calls a plotting routine, taken from the IMSL library (IMSL library, 1980), to output graphically the observed versus the predicted (least-squares fit) cell volume transients. Subroutine CELLCH uses a modified Adam's *predictor-corrector* method (see section 13.2.4) to integrate numerically the system of ordinary differential equations describing the transient behavior of the system. Subroutine ADFUN is called by CELLCH to perform, as required, the necessary computations, namely:

1. The calculation of the macroscopic phenomenological coefficients (subroutine BUILD) following relations 3.9 and 3.10.
2. The verification of the validity of the coefficients calculated above (subroutine CHECK) following the limits described by relations 2.26 and 2.27.
3. The calculation of the thermodynamic force gradients across the barrier (subroutine FGRAD) following equations 4.23 and 4.24, which are then used to compute the transmembrane fluxes of the permeable species (subroutine FLUXES) following equation 4.22.

4. The estimation of the change in cell volume (subroutine CVFUN) following equation 4.20.

5. The estimation of the change in cell membrane surface area (subroutine CAFUN) following the discussion of section 4.1.1.

6. Finally, the estimation of the change in cell solute concentrations (subroutine CCFUN) following equation 4.21.

```
C***********************************************************************
C
C  THIS FILE CONTAINS THE ROUTINES REFERRED TO AS "CELL BEHAVIOUR
C  ROUTINES" IN PROGRAM MCPERM. THEY ARE CALLED AND USED ESSENTIALLY
C  TO SIMULATE THE "THEORETICAL" BEHAVIOUR OF A CELL PUT INTO
C  CONTACT WITH AN OSMOTIC SOLUTION BY INTEGRATING A SYSTEM OF
C  ORDINARY DIFFERENTIAL EQUATIONS DESCRIBING THE PROCESS.
C
C  THE ROUTINES ARE:
C
C        INPUT  -USED TO INPUT THE DATA.
C       CELLCH  -USED AS DRIVER FOR THE INTEGRATION PROCESS.
C        ADFUN  -CALLED BY THE INTEGRATION ROUTINE ODE TO COMPUTE
C                THE DERIVATIVES.
C       FLUXES  -EVALUATES THE SOLVENT AND SOLUTE FLUXES ACROSS
C                THE CELL MEMBRANE.
C        FGRAD  -EVALUATES THE THERMODYNAMIC FORCE GRADIENTS
C                PRESENT IN THE SYSTEM.
C        CVFUN  -USED TO COMPUTE THE RELATIVE CELL VOLUME TIME
C                DERIVATIVE.
C        CAFUN  -USED TO COMPUTE THE TIME DERIVATIVE OF THE
C                DIMENSIONLESS CELL MEMBRANE SURFACE AREA.
C        CCFUN  -USED TO COMPUTE THE SPECIES DIMENSIONLESS
C                CELL CONCENTRATION TIME DERIVATIVES.
C        BUILD  -USED TO TRANSFORM ANY AM MATRIX COLUMN TO A FULL
C                MODIFIED PHENOMENOLOGICAL COEFFICIENT MATRIX.
C        CHECK  -VERIFIES IF THE THEORETICAL LIMITATIONS IMPOSED
C                ON THE PARAMETERS TO BE OPTIMIZED ARE RESPECTED.
C       OUTPUT  -OUTPUTS THE TABLE OF PREDICTED VS OBSERVED Y AT
C                THE OPTIMUM AND THEIR ASSOCIATED RESIDUALS.
C                GENERATES A PLOT OF THE EXPERIMENTAL AND THEORETICAL
C                DATA VERSUS TIME.
C
C***********************************************************************
C
C       **********   LATEST VERSION APRIL 2, 1985   **********
C                          BY CHRISTIAN TOUPIN.
C
C***********************************************************************
C                    CELL BEHAVIOUR SUBROUTINES.
C***********************************************************************
C
C  ROUTINE NAME  -INPUT
C
C  PURPOSE  -THIS SUBROUTINE IS CALLED TO INPUT THE DATA TO BE
C            USED BY THE PROGRAM.
C
C  USAGE  -CALL INPUT
C
C
      SUBROUTINE INPUT
C
C  DIMENSION TEMPV(T5),PMVV(T1+1),CSSOLM(T6,T1),CSIOV(T1),
C            PMM(T2,2),CASEM(T6,T5),YV(T4),
C            WTV(T4),XV(T4),AM(T2,T3),RESPV(T3),VOLV(T4).
C
      DIMENSION TEMPV(8),PMVV(3),CSSOLM(20,2),CSIOV(2),
     @          PMM(14,2),CASEM(20,8),YV(4000),XV(4000),
     @          WTV(4000),AM(14,103),RESPV(103),VOLV(4000)
C
      DOUBLE PRECISION R,TEMPV,PMVV,CSSOLM,CAREAO,VCO,RNOV,CSIOV,
     @                 PMM,YV,WTV,XV,TOLABS,TOLREL,TOLSPX,
     @                 TOLAVE,AM,RESPV,VOLV,RFTEMP
C
      INTEGER TEMPL,CONCL,SOLNUM,IMPNUM,SOLNP1,PCONUM,CASEM,NCOUNT,
     @        TESTSF,VMAX,VMAXP1,NCASES,DATAFL,ITEMAX
C
      COMMON /COPT1/ AM,RESPV
      COMMON /COPT2/ YV,WTV,XV,CASEM
      COMMON /COPT3/ VMAX,VMAXP1,SOLNUM,SOLNP1,IMPNUM,CONCL,TEMPL
      COMMON /COPT8/ TOLSPX,TOLAVE,TESTSF,ITEMAX
      COMMON /CCELL1/ PMVV,R,TEMPV,RFTEMP
      COMMON /CCELL2/ CSIOV,CSSOLM
      COMMON /CCELL3/ CAREAO,VCO,RNOV
      COMMON /CCELL4/ TOLABS,TOLREL
C
C
      WRITE(2,2250)
2250  FORMAT(////,' ',100('_'),//,' >>>>>>  DATA INPUT.',//)
C
C  INPUT DATA.
C
C  THE DATA FILE HAS TO CONTAIN THE REQUIRED INFORMATION IN
C  THE FOLLOWING FORM. ALL DATA ARE READ IN FREE FORMAT FORM.
C
      LOGICAL*1 FREE(1) /'*'/
C
C  DATA 'O INPUT.
C
C  1. SOLLTION CHARACTERISTICS.
C
C             R -UNIVERSAL GAS CONSTANT (MUST AGREE WITH THE UNIT
C                SYSTEM USED). IT IS SUGGESTED TO USE CGS SYSTEM.
C                THEREFORE R IS IN DYNE CM GMOLE-1 K-1.
C
C        RFTEMP -REFERENCE TEMPERATURE TO BE USED IN STUDIES OF
C                TEMPERATURE DEPENDENCE OF THE PERMEABILITY
C                COEFFICIENTS. WHEN ONLY ONE TEMPERATURE IS
C                CONSIDERED IN THE ANALYSIS, INPUT THAT TEMPERATURE
C                AS REFERENCE, IN CELSIUS.
C
      READ(1,FREE) R, RFTEMP
      WRITE(2,2000) R, RFTEMP
2000  FORMAT(/,' UNIVERSAL GAS CONSTANT: ',1PD10.3, /,
     @       ' REFERENCE TEMPERATURE: ', 0PF5.1)
C
C  TEMPL, TEMPV -NUMBER OF TEMPERATURES TO BE CONSIDERED IN THE
C                ANALYSIS (MAX=8) AND THEIR RESPECTIVE VALUES,
C                IN CELSIUS.
C
      READ(1,FREE) TEMPL, (TEMPV(K),K=1,TEMPL)
      WRITE(2,2010)
2010  FORMAT(/,' TEMPERATURE(S) TO BE CONSIDERED.',/,'  LEVEL INDEX'
     @      ,'     TEMPERATURE',/)
      DO 5 K=1,TEMPL
      WRITE(2,2015) K,TEMPV(K)
2015  FORMAT(7X,I1,13X,F5.1)
5     CONTINUE
C
```

```
C CONCL, SOLNUM -NUMBER OF CONCENTRATION LEVELS OF SAME SOLUTE(S)
C IMPNUM          TO BE CONSIDERED IN THE ANALYSIS (MAX=20), TOTAL
C                 NUMBER OF SOLUTES (MAX=2) AND TOTAL NUMBER OF
C                 IMPERMEABLE SOLUTES IN THE SYSTEM.
C
C                 **** IMPORTANT ****
C
C                 SOLUTES ARE HERE CONSIDERED AS EITHER INDEPENDENT
C                 COMPONENTS OR CAN REPRESENT A GROUP OF COMPONENTS
C                 BEHAVING IN A SIMILAR FASHION.
C
C                 IT IS CRUCIAL TO RESPECT THE FOLLOWING CONVENTIONS
C                 FOR THE INPUT OF SOLUTE RELATED DATA.
C
C                   1. THE PERMEABLE SOLUTES ARE INPUT FIRST, THE
C                      IMPERMEABLE SOLUTES LAST.
C
C                   2. THE DATA RELATED TO EACH SOLUTE MUST OCCUPY
C                      THE SAME INPUT POSITION SINCE THEY WILL BE
C                      ELEMENTS OF ARRAYS.
C
C                   3. A "CONCENTRATION LEVEL" REFERS EITHER TO AN
C                      OSMOTICUM COMPOSITION OR TO A REPLICATE OF A
C                      GIVEN OSMOTICUM.
C
C                   4. THE PROGRAM ASSUMES THAT THERE IS AN EQUAL
C                      NUMBER OF CONCENTRATION LEVELS AT EACH
C                      TEMPERATURE. THEREFORE, INPUT THE MAXIMUM
C                      NUMBER OF CONCENTRATION LEVELS TO BE
C                      ENCOUNTERED AT ANY TEMPERATURE AS CONCL.
C
C                 *******************
C
      READ(1,FREE) CONCL, SOLNUM, IMPNUM
      WRITE(2,2020) CONCL, SOLNUM, IMPNUM
2020  FORMAT(/,' TOTAL NUMBER OF CONCENTRATION LEVELS (INCLUDING',
     &        ' REPLICATES) TO BE CONSIDERED: ',I3,/,
     &        ' TOTAL NUMBER OF DIFFERENT SOLUTES: ',I3,/,
     &        ' TOTAL NUMBER OF IMPERMEABLE SOLUTES: ',I3)
C
C
C           PMVV -PARTIAL MOLAR VOLUME OF THE SOLVENT (WATER) AND
C                 OF THE SOLUTES PRESENT IN THE SYSTEM. INPUT ORDER:
C                 VW, VS1, VS2, ..., VSSOLNUM, IN CM-3 GMOLE.
C
      SOLNP1=SOLNUM+1
      READ(1,FREE) (PMVV(K),K=1,SOLNP1)
      WRITE(2,2030)
2030  FORMAT(/,' PARTIAL MOLAP VOLUME OF SOLVENT AND SOLUTES  ',/)
      DO 15 K=1,SOLNP1
      IF (K .NE. 1) GO TO 16
      WRITE(2,2260) PMVV(K)
2260  FORMAT(5X,'VW',5X,F8.3)
      GO TO 15
16    CONTINUE
      KM1=K-1
      WRITE(2,2270) KM1, PMVV(K)
2270  FORMAT(5X,'VS(',I1,')',2X,F8.3)
15    CONTINUE
C
C         CSSOLM -MATRIX OF OSMOTICUM SOLUTE CONCENTRATIONS, IN
C                 GMOLE CM-3.
C
C                 **** IMPORTANT ****
C
C                 THE CONCENTRATION OF SOLUTES MUST BE GIVEN IN
C                 TERMS OF MOLES OF OSMOTIC ACTIVE MATERIAL PER
C                 CM3 OF SOLUTION, I.E., IF THE SOLUTE DISSOCIATES,
C                 THE CONCENTRATION IS M * Z, THAT IS, THE
C                 CONCENTRATION OF THE UNDISSOCIATED SOLUTE (M)
C                 TIMES THE NUMBER OF MOLECULAR FRAGMENTS (Z).
C
C                 SINCE THE SOLUTIONS ARE ASSUMED TO BE IDEAL, THE
C                 CONCENTRATION DATA SHOULD BE GIVEN IN A FORM WHICH
C                 IS CONSISTENT WITH VAN'T HOFF EQUATION, I.E.
C
C                              CONC(I) = OP(I) /  RT
C
C                 WHERE OP(I) IS THE SOLUTION OSMOTIC PRESSURE DUE
C                 TO COMPONENT I AND RT IS THE UNIVERSAL GAS
C                 CONSTANT MULTIPLIED BY THE TEMPERATURE OF THE
C                 SYSTEM.
C
C                 SEE DESCRIPTION OF CSIOV BELOW FOR SPECIAL
C                 TREATMENT OF IMPERMEABLE SOLUTES.
C
C                 *******************
C
      WRITE(2,2040)
2040  FORMAT(/,' OSMOTICUM SOLUTE CONCENTRATIONS.',/,'  LEVEL INDEX'
     &       ,'  SOLUTE INDEX     CONCENTRATION',/)
      DO 10 I=1,CONCL
      READ(1,FREE) (CSSOLM(I,J),J=1,SOLNUM)
10    CONTINUE
      DO 20 I=1,CONCL
      DO 20 J=1,SOLNUM
      WRITE(2,2050) I, J, CSSOLM(I,J)
2050  FORMAT(6X,I2,13X,I1,11X,1PD10.3)
20    CONTINUE
C
C  2. CELL CHARACTERISTICS.
C
C    CAREAO, VCO -INITIAL CELL MEMBRANE SURFACE AREA (IN CM2),
C    RNOV         INITIAL CELL VOLUME (IN CM3) AND APPARENT
C                 NON-OSMOTIC CELL VOLUME (DIMENSIONLESS).
C
C                 THE LATTER VALUE IS A MEASURE OF THE FRACTION
C                 OF THE TOTAL CELLULAR VOLUME THAT CAN BE
C                 CONSIDERED TO BE OSMOTICALLY INACTIVE. ASSUMED
C                 TO BE CONSTANT AND TEMPERATURE INDEPENDENT.
C
      READ(1,FREE) CAREAO, VCO, RNOV
      WRITE(2,2060) CAREAO, VCO, RNOV
2060  FORMAT(/,' INITIAL CELL MEMBRANE SURFACE AREA: ',1PD10.3,/,
     &        ' INITIAL CELL VOLUME: ',D10.3,/,
     &        ' RELATIVE NON-OSMOTIC VOLUME:      ',0PF8.4)
C
C          CSIOV -VECTOR OF CELL SOLUTE CONCENTRATIONS, IN GMOLE
C                 CM-3.
C
```

```
C                      **** IMPORTANT ****
C
C                      IT IS ASSUMED THAT INITIALLY, THE ONLY SOLUTE
C                      PRESENT IN THE CELL IS A FICTITIOUS IMPERMEABLE
C                      SPECIES WHICH IS RESPONSIBLE FOR THE INITIAL CELL
C                      OSMOTIC PRESSURE.
C
C                      IN THE CASE WHERE THE OSMOTICUM CONTAINS ONLY
C                      IMPERMEABLE SOLUTES, IT IS SUGGESTED TO TREAT
C                      THEM AS ONE SPECIES SIMILAR TO THE CELL FICTITIOUS
C                      COMPONENT.
C
C                      DUE TO PROGRAM RESTRICTIONS, INPUT, FOR PERMEABLE
C                      SPECIES NOT PRESENT INITIALLY IN THE CELL, A VERY
C                      SMALL CONCENTRATION VALUE (.LE. 1.0E-05). SEE
C                      CSSOLM DESCRIPTION FOR MORE DETAILS.
C
C                      ******************
C
      READ(1,FREE) (CSIOV(K),K=1,SOLNUM)
      WRITE(2,2070)
2070  FORMAT(/,' INITIAL CELL SOLUTE CONCENTRATIONS ',/,
     &       '    SOLUTE INDEX     'CONCENTRATION',/)
      DO 25 K=1,SOLNUM
      WRITE(2,2080) K, CSIOV(K)
2080  FORMAT(8X,I1,11X,1PD10.3)
25    CONTINUE
C
C          PCONUM -TOTAL NUMBER OF COEFFICIENTS NECESSARY TO DESCRIBE
C                  THE PERMEABILITY OF THE CELL MEMBRANE.
C
C                  THIS NUMBER IS DEPENDENT ON THE NATURE OF THE
C                  SOLUTES PRESENT IN THE SYSTEM AND ON A POSSIBLE
C                  TEMPERATURE DEPENDENCE OF THE COEFFICIENTS.
C
C                    1. WHEN TEMPL IS SET TO 1:
C
C                     PCONUM = .5*(SOLNUM-IMPNUM+1)*(SOLNUM-IMPNUM+2)
C
C                    2. WHEN TEMPL IS .GE. 2:
C
C                     PCONUM = (SOLNUM-IMPNUM+1)*(SOLNUM-IMPNUM+2)
C
C             PMM -INITIAL GUESS OF THE VALUES OF THE CELL MEMBRANE
C                  MACROSCOPIC PERMEABILITY COEFFICIENTS RELATED TO
C                  EACH PERMEABLE SPECIES PRESENT IN THE SYSTEM.
C
C                  **** IMPORTANT ****
C
C                  INPUT SHOULD BE IN THE FOLLOWING FORM SO AS TO
C                  PERMIT TRANSFORMATION INTO A FULL SYMMETRICAL
C                  MATRIX OF COEFFICIENTS. INPUT ORDER MUST BE IN
C                  AGREEMENT WITH PRECEDING INPUT OF PARTIAL MOLAR
C                  VOLUMES.
C
C                    1. WHEN TEMPL IS SET TO 1, INPUT THE
C                       COEFFICIENTS AS FOLLOWS (1 COEFF. PER LINE):
C
C                                     PWW
C                                     PS1W
C                                     PS1S1
C                                     ....
C                                     P(SOLNUM-IMPNUM)W
C                                     ....
C                                     P(SOLNUM-IMPNUM)(SOLNUM-IMPNUM)
C
C                    2. WHEN TEMPL IS .GE. 2, THE APPARENT TRANSFER
C                       ENERGIES MUST BE INPUT ALONG WITH THE
C                       TEMPERATURE INDEPENDENT ESTIMATES OF THE
C                       MACROSCOPIC PERMEABILITY COEFFICIENTS
C                       (2 COEFF. PER LINE):
C
C                                  PWW       AWW
C                                  PS1W      AS1W
C                                  PS1S1     AS1S1
C                                  ....      ....
C
C                       THE PERMEABILITIES ARE IN GMOLE DYNE-1
C                       SEC-1, THE TRANSFER ENERGIES IN DYNE CM
C                       GMOLE-1.
C
C  THE MATRIX OF PARAMETER VALUES TO BE USED IN THE OPTIMIZATION
C  PROCESS (AM MATRIX) IS INITIALIZED SIMULTANEOUSLY.
C
      READ(1,FREE) PCONUM
      VMAX=PCONUM
      VMAXP1=VMAX+1
      WRITE(2,2090)
2090  FORMAT(/,' INITIAL GUESS OF CELL MEMBRANE MACROSCOPIC',
     @       ' PERMEABILITY COEFFICIENTS ',//,'  PERMEABILITY',
     @       ' COEFFICIENT  APPARENT TRANSFER ENERGY',/)
      IF (TEMPL .NE. 1) GO TO 50
      DO 30 I=1,PCONUM
      READ(1,FREE) PMM(I,1)
      WRITE(2,2100) PMM(I,1)
2100  FORMAT(8X,1PD10.3)
      AM(I,1)=PMM(I,1)
30    CONTINUE
      GO TO 60
50    CONTINUE
      PCONUM=PCONUM/2
      DO 40 I=1,PCONUM
      READ(1,FREE) PMM(I,1), PMM(I,2)
      WRITE(2,2110) PMM(I,1), PMM(I,2)
2110  FORMAT(8X,1PD10.3,17X,D10.3)
      AM(I,1)=PMM(I,1)
      AM(PCONUM+I,1)=PMM(I,2)
40    CONTINUE
60    CONTINUE
C
C  3. EXPERIMENTAL DATA.
C
C         DATAFL -FLAG TO INDICATE THE NATURE OF THE EXPERIMENTAL
C                 DATA TO BE READ. IF SET TO 0, CELL VOLUMES IN
C                 VOLUME UNITS, I.E., IN CM3, ARE GIVEN. IF SET
C                 TO 1, RELATIVE CELL VOLUME VALUES ARE PROVIDED.
C
C          CASEM -MATRIX IN WHICH EACH ELEMENT REPRESENTS THE NUMBER
C                 OF CASES FOR A PARTICULAR TEMPERATURE AND
C                 CONCENTRATION LEVEL COMBINATION. FOR MISSING
```

```
C                    COMBINATION, INPUT 0.
C
C             VOLV  -VECTOR OF EXPERIMENTAL CELL VOLUME VALUES, IN
C                    CM3.
C
C               YY  -VECTOR OF EXPERIMENTAL RELATIVE CELL VOLUMES.
C
C              WTY  -VECTOR OF THEIR ASSOCIATED WEIGHTS.
C
C                    THE WEIGHTS ARE THE INVERSE OF THE ESTIMATED
C                    VARIANCES OF THE SAMPLES IF AVAILABLE. OTHERWISE
C                    ANY MEANINGFUL MEASURE OF THE PRECISION OF THE
C                    EXPERIMENTAL DATA CAN BE USED.
C
C               XV  -VECTOR OF INDEPENDENT VARIABLE VALUE (TIME),
C                    IN SEC.
C
C                    *********** IMPORTANT ***********
C
C                    DUE TO CERTAIN PROGRAM RESTRICTIONS, THE
C                    EXPERIMENTAL DATA MUST BE INPUT AS FOLLOWS.
C
C                       1. FOR A GIVEN TEMPERATURE LEVEL, INPUT IN
C                          SEQUENCE THE SET OF EXPERIMENTAL VALUES
C                          CORRESPONDING TO THE FIRST CONCENTRATION
C                          LEVEL, THEN THE DATA CORRESPONDING TO THE
C                          SECOND AND SO ON. ONCE ALL CONCENTRATION
C                          LEVELS HAVE BEEN COVERED, INPUT DATA
C                          CORRESPONDING TO THE SECOND TEMPERATURE
C                          LEVEL AND SO ON UNTIL ALL TEMPERATURES ARE
C                          COVERED. FOR MISSING COMBINATIONS, DATA ARE
C                          NOT REQUIRED SINCE, IN THAT CASE, CASEM(I,J)
C                          FOR THESE COMBINATIONS HAVE BEEN SET TO 0.
C
C                       2. THE FIRST LINE OF EACH COMBINATION DATA SET
C                          (INCLUDING MISSING COMBINATIONS) MUST CONTAIN
C                          THE NUMBER OF CASES FOR THAT SET.
C
C                       3. THE FOLLOWING LINES CONTAIN THE CELL VOLUME
C                          VALUES IN RELATIVE OR ABSOLUTE FORM, THEIR
C                          ASSOCIATED WEIGHTS AND THE TIME AT WHICH
C                          THE MEASUREMENTS WERE MADE (1 CASE PER LINE)
C
C                       4. NOTE THAT A GRAND TOTAL OF 4000 CASES CAN BE
C                          INPUT WITH A MAXIMUM OF 120 CASES PER
C                          TEMPERATURE-CONCENTRATION LEVEL COMBINATION.
C
C                       5. BECAUSE OF THE ASSUMPTION THAT ALL THE CELLS
C                          HAVE THE SAME INITIAL VOLUME, THE VOLUME
C                          VALUES AT TIME EQUAL 0 ARE SET EXPLICITLY
C                          BY THE PROGRAM (FOR ALL SETS) TO THE INITIAL
C                          CELL VOLUME VALUE INPUT PREVIOUSLY.
C
C                    ********************************
C
C
      WRITE(2,2120)
2120  FORMAT(////,' EXPERIMENTAL DATA SETS TO BE CONSIDERED ',/)
      READ(1,FREE) DATAFL
      IF (DATAFL .EQ. 1) WRITE(2,2122)
      IF (DATAFL .EQ. 0) WRITE(2,2123)
2122  FORMAT(' *** VOLUME VALUES ARE GIVEN IN RELATIVE FORM ***',/)
2123  FORMAT(' *** VOLUME VALUES ARE GIVEN IN ABSOLUTE FORM ***',/)
      WRITE(2,2124) VCO
2124  FORMAT(' *** INITIAL VOLUMES ARE SET EXPLICITLY TO ',
     @        1PD10.3,'  ***',/)
      NCOUNT=1
      DO 70 J=1,TEMPL
      DO 70 I=1,CONCL
      READ(1,FREE) CASEM(I,J)
      IF (CASEM(I,J) .EQ. 0) GO TO 70
      WRITE(2,2130) J, I, CASEM(I,J)
2130  FORMAT(//,'  TEMPERATURE LEVEL: ',I2,7X,'CONCENTRATION LEVEL: ',
     @        I2,/,'  NUMBER OF CASES: ',I4)
      WRITE(2,2140)
2140  FORMAT(//,'  CELL VOLUMES    RELATIVE CELL VOLUMES',
     @               ASSOCIATED WEIGHTS    TIME',/)
C
      NCASES=CASEM(I,J)
      DO 70 K=1,NCASES
      IF (DATAFL .EQ. 0) GO TO 85
      READ(1,FREE) YV(NCOUNT), WTV(NCOUNT), XV(NCOUNT)
      IF (K .EQ. 1) YV(NCOUNT)=1.0D0
      VOLV(NCOUNT)=YV(NCOUNT)*VCO
      GO TO 87
85    CONTINUE
      READ(1,FREE) VOLV(NCOUNT), WTV(NCOUNT), XV(NCOUNT)
      IF (K .EQ. 1) VOLV(NCOUNT)=VCO
      YV(NCOUNT)=VOLV(NCOUNT)/VCO
87    CONTINUE
      WRITE(2,2220) VOLV(NCOUNT), YV(NCOUNT), WTV(NCOUNT),
     @               XV(NCOUNT)
2220  FORMAT(3X,1PD10.3,12X,0PF8.4,16X,1PD10.3,7X,0PF8.2)
      NCOUNT=NCOUNT+1
70    CONTINUE
C
C  4. CONTROL VARIABLES.
C
C TOLABS, TOLREL -TOLERANCES FOR ERROR CONTROL IN ADAM'S ALGORITHM.
C                 INPUT VALUES SUGGESTED:
C
C                               0.0   FOR ABSOLUTE ERROR TEST
C                           1.0E-04   FOR RELATIVE ERROR TEST
C
C                 (SEE SUBROUTINE CELLCH AND ODE FOR DETAILS).
C
      READ(1,FREE) TOLABS, TOLREL
      WRITE(2,2150)
2150  FORMAT(////,' TOLERANCES FOR ERROR CONTROL IN ADAM''S',
     @         ' ALGORITHM.',/)
      WRITE(2,2230) TOLABS, TOLREL
2230  FORMAT('  ABSOLUTE ERROR TEST: ',1PD10.3,/,
     @        '  RELATIVE ERROR TEST: ',D10.3)
C
C  TOLSPX, TOLAVE -STARTING TOLERANCES FOR HALTING FIRST SIMPLEX
C                  SEARCH. INPUT VALUES SUGGESTED:
C
C                    0.01   AS LIMIT VALUE OF THE COEFFICIENT OF
C                           VARIATION OF THE RESPONSES IN ANY
C                           SIMPLEX..
```

```
C
C                    0.01    AS LIMIT VALUE OF THE RELATIVE DIFFERENCE
C                            BETWEEN THE AVERAGE RESPONSES OF TWO
C                            CONSECUTIVE SIMPLICES.
C
C                  (SEE SUBROUTINE MODSPX FOR DETAILS).
C
C            TESTSF -THE USER CAN ACCESS THE VALUES OF THE TEST
C                  VARIABLES CALCULATED DURING A SIMPLEX SEARCH
C                  (AVERT AND SPXT). IF LISTING DESIRED, SET
C                  TESTSF TO 1, OTHERWISE TESTSF IS SET TO 0
C                  (SEE SUBROUTINE MODSPX FOR DETAILS).
C
C            ITEMAX -MAXIMUM NUMBER OF ITERATIONS ALLOWED TO FIND
C                  AN OPTIMUM IN ANY GIVEN SIMPLEX. IT IS SUGGESTED
C                  TO SET THIS VALUE TO 25.
C
      READ(1,FREE) TOLSPX, TOLAVE, TESTSF, ITEMAX
      WRITE(2,2160)
2160  FORMAT(///,' TEST VALUES FOR HALTING FIRST SIMPLEX SEARCH.',/)
      WRITE(2,2240) TOLSPX, TOLAVE
2240  FORMAT('  COEFFICIENT OF VARIATION: ',1PD10.3,/,
     $        '  RELATIVE DIFFERENCE BETWEEN AVERAGES: ',D10.3)
      IF (TESTSF .EQ. 1) GO TO 80
      WRITE(2,2170)
2170  FORMAT(///,' *** NO LISTING OF TEST VARIABLES ***')
      GO TO 90
80    CONTINUE
      WRITE(2,2180)
2180  FORMAT(///,' *** LISTING OF TEST VARIABLES PROVIDED ***')
90    CONTINUE
      WRITE(2,2182) ITEMAX
2182  FORMAT(//,' *** A MAXIMUM OF ',I5,' ITERATIONS IS ALLOWED',
     $        ' IN ANY SIMPLEX SEARCH ***')
C
C
      WRITE(2,2190) VMAX
2190  FORMAT(////,' NUMBER OF PARAMETERS TO OPTIMIZE: ',I4)
      WRITE(2,2200)
2200  FORMAT(/,' INITIAL COEFFICIENTS IN PARAMETER MATRIX.',/)
      DO 100 I=1,VMAX
      WRITE(2,2210) I, AM(I,1)
2210  FORMAT('   ROW (',I2,')',6X,1PD11.4)
100   CONTINUE
      WRITE(2,2260)
2260  FORMAT(//,' ',100('_'))
      RETURN
      END
C
C
C***********************************************************************
C
C  ROUTINE NAME  -CELLCH
C
C  PURPOSE  -USES A MODIFIED ADAMS PREDICTOR-CORRECTOR METHOD
C            TO EVALUATE, AS TIME PASSES, THE CHANGE IN CELL VOLUME
C            AND SOLUTE CONCENTRATIONS IN A TYPICAL CELL WHEN THIS
C            CELL IS PUT INTO CONTACT WITH AN OSMOTICUM.
C
C            THE ROUTINE MAKES USE OF THE THEORY OF IRREVERSIBLE
C            THERMODYNAMICS TO CHARACTERIZE THE CROSS MEMBRANE
C            FLUXES.
C
C            THE DIFFERENTIAL EQUATIONS USED IN THE CALCULATION
C            OF THESE CHANGES HAVE BEEN DIMENSIONALIZED. THIS
C            IMPLIES THAT ALL CONC. AND VOLUME VALUES ARE DIMENSION-
C            LESS. THE INTEGRATION IS NEVERTHELESS PERFORMED ON A
C            REAL TIME BASIS.
C
C  USAGE  -CALL CELLCH(CALCYV)
C
C  ARGUMENTS AND VARIABLES
C
C             CALCYV -VECTOR OF CALCULATED THEORETICAL RELATIVE CELL
C                     VOLUME VALUES.
C             RATIOV -VECTOR OF FLUX RATIOS OF SYSTEM SPECIES.
C              NCSFV -VECTOR OF CONCENTRATION NORMALIZATION FACTORS
C                     USED TO GENERATE DIMENSIONLESS CONC. VALUES.
C             NCSSLV -VECTOR OF DIMENSIONLESS OSMOTICUM SOLUTE
C                     CONCENTRATIONS.
C               TIME -CURRENT REAL TIME, IN SEC.
C              TSTEP -TOTAL NUMBER OF INTEGRATION STEPS TO BE DONE
C                     TO REACH FINAL TIME.
C             NCOUNT -POSITION COUNTER.
C             CCALCY -POSITION COUNTER. USED TO SEND DATA TO CALCYV.
C              FLUXV -VECTOR OF FLUX VALUES AS COMPUTED IN ADFUN.
C
C  PROGRAMMING NOTES
C
C    SUBROUTINE ODE IS CALLED TO PERFORM THE INTEGRATION OF THE
C    DIFFERENTIAL EQUATIONS DESCRIBING THE BEHAVIOUR OF THE STUDIED
C    CELL. SEE ROUTINE FOR DETAILS ON THE ALGORITHM AND ON ITS
C    USAGE.
C
C
      SUBROUTINE CELLCH(CALCYV)
C
C
C  DIMENSION CALCYV(T7),YV(T4),WTV(T4),CASEM(T6,T6),PMVV(T1+1),
C           TEMPV(T5),CSIOV(T1),CSSOLM(T6,T1),XV(T4),
C           FLUXV(T1+2),NCSFV(T1),NCSSLV(T1),Y(T1+2),
C           WORK(100+((T1+2)*21)),IWORK(5),RATIOV(T1+1).
C
      DIMENSION CALCYV(120),YV(4000),WTV(4000),CASEM(20,6),
     $          PMVV(3),TEMPV(6),CSIOV(2),CSSOLM(20,2),
     $          XV(4000),FLUXV(4),NCSFV(2),NCSSLV(2),Y(4),
     $          WORK(184),IWORK(5),RATIOV(3)
C
      DOUBLE PRECISION CALCYV,YV,WTV,XV,PMVV,R,TEMPV,CSIOV,CSSOLM,
     $                 CAREAO,VCO,RHOV,TOLABS,TOLREL,FLUXV,
     $                 NCSFV,NCSSLV,Y,TIME,RELERR,ABSERR,X,
     $                 XEND,WORK,RATIOV,RFTEMP
C
      INTEGER CASEM,VMAX,VMAXP1,SOLNUM,SOLNP1,IMPNUM,CONCL,TEMPL,
     $        CHECKF,ADF,TIND,CLIND,AMCIND,SOLNP2,TSTEP,
     $        NEQN,IFLAG,CCALCY,KAD,IWORK,NCTIND,NCOUNT
C
C  EXECUTION-TIME FORMAT STATEMENTS.
C  DIMENSION DIGIT(T1+3)/'1',....,'T1+3'/
C
```

```
      INTEGER DIGIT(5)/'1','2','3','4','5'/
      INTEGER VF(4)/'(','    ',' F8.','4)'/
C
      EXTERNAL ADFUN
C
      COMMON /COPT2/ YV,WTV,XV,CASEM
      COMMON /COPT3/ VMAX,VMAXP1,SOLNUM,SOLNP1,IMPNUM,CONCL,TEMPL
      COMMON /COPT5/ CHECKF,ADF
      COMMON /CCELL1/ PMVV,R,TEMPV,RFTEMP
      COMMON /CCELL2/ CSIOV,CSSOLM
      COMMON /CCELL3/ CAREAO,VCO,RNOV
      COMMON /CCELL4/ TOLABS,TOLREL
      COMMON /CCELL5/ TIND,CLIND,AMCIND,NCTIND
      COMMON /CCELL7/ FLUXV,NCSFV,NCSSLV
C
C
C
C  EVALUATE CONCENTRATION NORMALIZATION FACTORS AND THE NORMALIZED
C  OSMOTICUM CONCENTRATIONS.
C
      DO 10 K=1,SOLNUM
      NCSFV(K)=(CSIOV(K)+CSSOLM(CLIND,K))/2.0D0
10    NCSSLV(K)=CSSOLM(CLIND,K)/NCSFV(K)
C
C  DEFINE INITIAL PARAMETER VALUES. THE ELEMENTS OF Y VECTOR ARE
C  DEFINED AS FOLLOWS (AT ANY TIME):
C
C        Y(1) TO Y(SOLNUM): SOLUTE DIMENSIONLESS CONCENTRATIONS
C                           IN THE CELL.
C                Y(SOLNP1): RELATIVE CELL VOLUME.
C                Y(SOLNP2): RELATIVE CELL MEMBRANE SURFACE AREA
C
      SOLNP2=SOLNUM+2
      DO 20 K=1,SOLNUM
20    Y(K)=CSIOV(K)/NCSFV(K)
      Y(SOLNP1)=1.0D0
      Y(SOLNP2)=1.0D0
      CALCYV(1)=1.0D0
      TIME=0.0D0
      TSTEP=CASEM(CLIND,TIND)-1
      NCOUNT=NCTIND
C
C  DEFINE CONTROLLING PARAMETERS NEEDED BY SUBR ODE.
C
      NEQN=SOLNP2
      IFLAG=1
C
C  A MIXED ERROR CONTROL IS PERFORMED (SEE CODE FOR DETAILS).
C
      RELERR=TOLREL
      ABSERR=TOLABS
C
C  INITIAL INDEPENDENT VAR. VALUE (TIME).
C
      X=TIME
C
C  INITIALIZE I/O UNIT AND COUNTERS.
C
C     REWIND3
      CCALCY=2
C
C  PERFORM INTEGRATION UNTIL FINAL TIME REACHED.
C
      DO 40 KAD=1,TSTEP
C
C  SET TIME VALUE AT WHICH SOLUTION IS DESIRED.
C
      XEND=XV(NCOUNT+1)
C
C  CALL THE INTEGRATION SUBROUTINE.
C
      ADF=0
110   CALL ODE(ADFUN,NEQN,Y,X,XEND,RELERR,ABSERR,IFLAG,WORK,IWORK)
C
C  IF CHECKF HAS BEEN SET TO 1, END PROCEDURE AND GO BACK TO
C  CALLING ROUTINE.
C
      IF (CHECKF .EQ. 1) GO TO 90
C
C  TEST FOR SIGNAL ERROR OR MALFUNCTION OF THE ROUTINE.
C
      IF (IFLAG .EQ. 2) GO TO 60
C
C  IF THE ACCURACY REQUESTED IS TOO HIGH, CALL AGAIN THE ROUTINE
C  WITH THE OPTIMAL TOLERANCES COMPUTED INTERNALLY (SEE CODE FOR
C  DETAILS).
C
      IF (IFLAG .NE. 3) GO TO 100
      WRITE(2,2010) RELERR,ABSERR
2010  FORMAT(/,' RELERR, ABSERR SUGGESTED: ',1P2D10.3)
      GO TO 110
100   CONTINUE
      WRITE(2,2000) IFLAG
2000  FORMAT(//,' ***** MALFUNCTION OF SUBR. ODE *****. IFLAG: ',I4)
      ADF=1
      GO TO 90
60    CONTINUE
C
C  OTHERWISE GET CURRENT RELATIVE CELL VOLUME VALUE.
C
C  BUILD CALCYV. GET SOLUTE CONCENTRATIONS AND FLUX RATIOS AS WELL.
C
      CALCYV(CCALCY)=Y(SOLNP1)
      DO 80 K=1,SOLNP1
80    RATIOV(K)=(FLUXV(K)*PMVV(K)*Y(SOLNP2)*CAREAO)/FLUXV(SOLNP2)
      TIME=XEND
      VF(2)=DIGIT(SOLNP2+1)
C     WRITE(3,VF) TIME,(Y(K), K=1,SOLNP2)
      VF(2)=DIGIT(SOLNP1)
C     WRITE(3,VF) (RATIOV(K), K=1,SOLNP1)
      CCALCY=CCALCY+1
      NCOUNT=NCOUNT+1
40    CONTINUE
90    RETURN
      END
C
C
C ***********************************************************
C
C  ROUTINE NAME  -ADFUN
```

```
C
C   PURPOSE  -THIS SUBROUTINE IS CALLED BY ODE TO PERFORM THE
C             SIMULTANEOUS EVALUATION OF THE TIME DERIVATIVE FUNC.
C             ON INPUT IT RECEIVES THE VALUES NEEDED TO EVALUATE THE
C             FUNCTIONS, ON OUTPUT IT GIVES THE VALUES OF THE TIME
C             DERIVATIVES.
C
C   USAGE   -CALL ADFUN(X,Y,YPRIME,ISTOP)
C
C   ARGUMENTS AND VARIABLES
C
C                      X -CURRENT VALUE OF THE INDEPENDENT VARIABLE.
C                      Y -VECTOR OF CURRENT VALUES OF THE DEPENDENT
C                         VARIABLES.
C                 YPRIME -VECTOR OF THE FIRST DERIVATIVES OF Y WITH
C                         RESPECT TO X.
C                  ISTOP -FLAG TO INDICATE THAT THE INTEGRATION PROCESS
C                         MUST BE STOPPED SINCE THE PARAMETER VALUES
C                         ARE INVALID. IN THAT CASE ISTOP IS SET TO 1.
C                 SAVGCV -VECTOR OF THE CURRENT SOLUTE AVERAGE CONC.
C                         (GMOLE CM-3).
C                  CVFUN -FUNCTION SUBPROGRAM TO EVALUATE THE CELL
C                         VOLUME TIME DERIVATIVE.
C                  CAFUN -FUNCTION SUBPROGRAM TO EVALUATE THE TIME
C                         DERIVATIVE OF THE DIMENSIONLESS CELL
C                         MEMBRANE SURFACE AREA.
C                  CCFUN -FUNCTION SUBPROGRAM TO EVALUATE THE CELL
C                         INTERNAL CONCENTRATION TIME DERIVATIVE FOR
C                         ANY GIVEN SPECIES.
C                  FLUXV -VECTOR OF FLUX VALUES (GMOLE CM-2 SEC-1).
C                 NFLUXV -VECTOR OF NORMALIZED FLUX VALUES.
C
C
      SUBROUTINE ADFUN(X,Y,YPRIME,ISTOP)
C
C
C   DIMENSION Y(T1+2),YPRIME(T1+2),FLUXV(T1+2),NFLUXV(T1+1),
C            NCSFV(T1),NCSSLV(T1),SAVGCV(T1),CSIOV(T1),CSSOLM(T6,T1)
C
      DIMENSION Y(4),YPRIME(4),FLUXV(4),NFLUXV(3),NCSFV(2),
     &          NCSSLV(2),SAVGCV(2),CSIOV(2),CSSOLM(20,2)
C
      DOUBLE PRECISION Y,YPRIME,FLUXV,X,CVFUN,CCFUN,NFLUXV,NCSFV,
     &                 NCSSLV,SAVGCV,CSIOV,CSSOLM,CAFUN,CAREAO,VCO,
     &                 RNOV
C
      INTEGER VMAX,VMAXP1,SOLNUM,SOLNP1,IMPNUM,CONCL,TEMPL,CHECKF,
     &        ADF,ISTOP,TIND,CLIND,AMCIND,SOLNP2,NCTIND
C
      COMMON /COPT3/ VMAX,VMAXP1,SOLNUM,SOLNP1,IMPNUM,CONCL,TEMPL
      COMMON /COPT6/ CHECKF,ADF
      COMMON /CCELL2/ CSIOV,CSSOLM
      COMMON /CCELL3/ CAREAO,VCO,RNOV
      COMMON /CCELL6/ TIND,CLIND,AMCIND,NCTIND
      COMMON /CCELL7/ FLUXV,NCSFV,NCSSLV
C
C   EVALUATE THE SOLUTE AVERAGE CONCENTRATIONS AND CHECK IF
C   THE MODIFIED PHENOME. COEFF. TO BE USED IN THE COMING CALC.
C   ARE VALID (MONSAM MATRIX). IF NOT END PROCEDURE AND GO BACK TO
C   CALLING ROUTINE.
C
      DO 10 K=1,SOLNUM
10    SAVGCV(K)=((Y(K)*NCSFV(K))+CSSOLM(CLIND,K))/2.0D0
C
C   BUILD MONSAM MATRIX TO BE USED IN THE FLUX CALCULATIONS.
C
      CALL BUILD(SAVGCV)
      ISTOP=0
      CALL CHECK
C
C   IF FLAG HAS BEEN SET TO 1, STOP THE INTEGRATION PROCESS AND
C   RETURN TO THE CALLING ROUTINE.
C
      IF (CHECKF .NE. 1) GO TO 40
      ISTOP=1
      RETURN
40    CONTINUE
C
C   OTHERWISE PERFORM THE EVALUATION OF THE DERIVATIVES.
C
C   EVALUATE FIRST THE SOLVENT AND SOLUTES NORMALIZED FLUXES ACROSS
C   THE CELL MEMBRANE.
C
      CALL FLUXES(Y,NFLUXV)
C
C   THEN EVALUATE THE TIME DERIVATIVES AND DEFINE YPRIME ELEMENTS.
C
C   CHANGE IN CELL VOLUME.
C
      SOLNP2=SOLNUM+2
      YPRIME(SOLNP1)=CVFUN(NFLUXV,Y(SOLNP2))
C
C   CHANGE IN CELL MEMBRANE SURFACE AREA.
C
      YPRIME(SOLNP2)=CAFUN(Y(SOLNP1),YPRIME(SOLNP1))
C
C   CHANGE IN CELL SOLUTE CONCENTRATIONS.
C
      DO 20 K=1,SOLNUM
20    YPRIME(K)=CCFUN(Y(SOLNP1),Y(SOLNP2),Y(K),NCSFV(K),NFLUXV(K+1),
     &                YPRIME(SOLNP1))
C
C   DEFINE THE CURRENT VALUES OF THE FLUXES. NOTE THAT SINCE THE
C   EQUATIONS USED TO EVALUATE THESE FLUXES ARE NORMALIZED,
C   CORRECTION FACTORS ARE NEEDED TO HAVE FLUX VALUES THAT HAVE
C   DIMENSIONS (GMOLE CM-2 SEC-1).
C
C   FLUX OF SOLVENT (WATER).
C
      FLUXV(1)=NFLUXV(1)*VCO/CAREAO
C
C   FLUX OF SOLUTES.
C
      DO 30 K=2,SOLNP1
30    FLUXV(K)=NFLUXV(K)*VCO/CAREAO
C
C   CURRENT VALUE OF THE CELL VOLUME CHANGE, I.E. THE VOLUME FLUX
C   (CM3 SEC-1).
C
      FLUXV(SOLNP2)=YPRIME(SOLNP1)*VCO
      RETURN
      END
C
C
C ****************************************************************
C
C  ROUTINE NAME  -FLUXES
C
C  PURPOSE   -THIS SUBROUTINE IS USED TO EVALUATE THE SOLVENT AND
C             SOLUTE FLUXES ACROSS THE CELL MEMBRANE AS A FUNCTION
C             THE SOLUTE DIMENSIONLESS CONCENTRATIONS IN AND OUT THE
C             CELL. THIS SUBROUTINE MAKES USE OF THE MODIFIED
C             PHENOMENOLOGICAL COEFFICIENTS (MONSAM MATRIX)
C
C  USAGE   -CALL FLUXES(Y,NFLUXV)
C
C  ARGUMENTS AND VARIABLES
C
C                     Y -VECTOR OF THE CURRENT VALUES OF SOLUTE CELL
C                        DIMENSIONLESS CONCENTRATIONS.
C                NFLUXV -VECTOR OF NORMALIZED FLUX VALUES
C                FORCES -VECTOR OF DIMENSIONLESS THERMODYNAMIC
C                        FORCES.
C
C
C
       SUBROUTINE FLUXES(Y,NFLUXV)
C
C
C  DIMENSION NFLUXV(T1+1),FORCES(T1+1),MONSAM(T1+1,T1+1),Y(T1+2).
C
       DIMENSION NFLUXV(3),FORCES(3),MONSAM(3,3),Y(4)
C
       DOUBLE PRECISION NFLUXV,FORCES,MONSAM,Y
C
       INTEGER VMAX,VMAXP1,SOLNUM,SOLNP1,IMPNUM,CONCL,TEMPL
C
       COMMON /COPT3/ VMAX,VMAXP1,SOLNUM,SOLNP1,IMPNUM,CONCL,TEMPL
       COMMON /CCELL8/ MONSAM
C
C
C
C  EVALUATE THE DIMENSIONLESS FORCES USED TO CALCULATE THE SPECIES
C  FLUXES.
C
       CALL FGRAD(Y,FORCES)
C
C  GET THE VALUES OF THE FLUXES BY MULTIPLING THE MODIFIED PHENOME-
C  NOLOGICAL COEFF. MATRIX (MONSAM) BY THE VECTOR FORCES.
C
       DO 50 I=1,SOLNP1
       NFLUXV(I)=0.0D0
       DO 50 J=1,SOLNP1
50     NFLUXV(I)=NFLUXV(I)+MONSAM(I,J)*FORCES(J)
C
C  DUE TO THE DEFINITION OF A DIFFERENCE GRADIENT AS:
C
C      DELTA = QUANTITY INSIDE THE CELL - QUANTITY OUTSIDE THE CELL
C
C  AN EFFLUX IS POSITIVE WHILE AN INFLUX IS NEGATIVE. TO STAY IN
C  AGREEMENT WITH THE CONVENTION THAT AN EFFLUX IS NEGATIVE
C  (EQUIVALENT TO A LOSS) AND AN INFLUX IS POSITIVE (EQUIVALENT
C  TO A GAIN), THE SIGN OF THE FLUX VALUES MUST BE INVERTED.
C
C
       DO 60 K=1,SOLNP1
60     NFLUXV(K)=-NFLUXV(K)
       RETURN
       END
C
C
C ****************************************************************
C
C  ROUTINE NAME  -FGRAD
C
C  PURPOSE   -THIS SUBROUTINE IS USED TO EVALUATE THE THERMODYNAMIC
C             FORCE GRADIENTS PRESENT IN THE SYSTEM. THESE GRADIENTS
C             ARE RESPONSIBLE FOR THE OBSERVED FLUXES ACROSS THE
C             REGION OF INHOMOGENEITY.
C
C             NOTE THAT IT IS ASSUMED THAT NO HYDROSTATIC PRESSURE
C             GRADIENT IS PRESENT ACROSS THE BARRIER.
C
C  USAGE   -CALL FGRAD(Y,FORCES)
C
C  ARGUMENTS AND VARIABLES
C
C                     Y -VECTOR OF THE CURRENT VALUES OF SOLUTE CELL
C                        DIMENSIONLESS CONCENTRATIONS.
C                FORCES -VECTOR OF DIMENSIONLESS THERMODYNAMIC FORCES.
C                NSAVCV -VECTOR OF CURRENT DIMENSIONLESS SOLUTE AVERAGE
C                        CONCENTRATIONS.
C                   SUM -SUMMATION VARIABLE.
C
C
       SUBROUTINE FGRAD(Y,FORCES)
C
C
C  DIMENSION Y(T1+2),FORCES(T1+1),FLUXV(T1+2),NCSFV(T1),NCSSLV(T1),
C            PMVV(T1+1),NSAVCV(T1),TEMPV(T5).
C
       DIMENSION Y(4),FORCES(3),FLUXV(4),NCSFV(2),NCSSLV(2),PMVV(3),
     @           NSAVCV(2),TEMPV(6)
C
       DOUBLE PRECISION Y,FORCES,FLUXV,NCSFV,NCSSLV,PMVV,R,TEMPV,
     @                  SUM,NSAVCV,RFTEMP
C
       INTEGER VMAX,VMAXP1,SOLNUM,SOLNP1,IMPNUM,CONCL,TEMPL
C
       COMMON /COPT3/ VMAX,VMAXP1,SOLNUM,SOLNP1,IMPNUM,CONCL,TEMPL
       COMMON /CCELL1/ PMVV,R,TEMPV,RFTEMP
       COMMON /CCELL7/ FLUXV,NCSFV,NCSSLV
C
C
C  EVALUATE THE CURRENT DIMENSIONLESS SOLUTE AVERAGE CONCENTRATIONS.
C
       DO 30 K=1,SOLNUM
       NSAVCV(K)=(Y(K)+NCSSLV(K))/2.0D0
30     CONTINUE
C
C  EVALUATE THE SOLVENT GRADIENT.
```

```
C
      SUM=0.0D0
      DO 10 K=1,SOLNUM
      SUM=SUM+(NCSFV(K)*(Y(K)-NCSSLV(K)))
10    CONTINUE
      FORCES(1)=-PMVV(1)*SUM
C
C  EVALUATE THE SPECIES GRADIENTS.
C
      DO 20 K=2,SOLNP1
      FORCES(K)=(Y(K-1)-NCSSLV(K-1))/NSAVCV(K-1)
20    CONTINUE
      RETURN
      END
C
C
C*********************************************************************
C
C  ROUTINE NAME   -CVFUN
C
C  PURPOSE   -THIS FUNCTION SUBPROGRAM EVALUATES THE RELATIVE CELL
C             VOLUME TIME DERIVATIVE.
C
C             THE VOLUME CHANGE ON MIXING IS CONSIDERED NEGLIGIBLE.
C
C  USAGE   -XYZ=CVFUN(NFLUXV,YLL)
C
C  ARGUMENTS AND VARIABLES.
C
C       NFLUXV -VECTOR OF NORMALIZED FLUX VALUES.
C          YLL -CURRENT VALUE OF THE RELATIVE CELL MEMBRANE SURFACE
C               AREA.
C          SUM -SUMMATION VARIABLE.
C
C
      DOUBLE PRECISION FUNCTION CVFUN(NFLUXV,YLL)
C
C
C  DIMENSION NFLUXV(T1+1),PMVV(T1+1),TEMPV(T6).
C
      DIMENSION NFLUXV(3),PMVV(3),TEMPV(6)
C
      DOUBLE PRECISION NFLUXV,YLL,PMVV,R,TEMPV,SUM,RFTEMP,RT,
     @                 RTRF
C
      INTEGER VMAX,VMAXP1,SOLNUM,SOLNP1,IMPNUM,CONCL,TEMPL
C
      COMMON /COPT3/ VMAX,VAMXP1,SOLNUM,SOLNP1,IMPNUM,CONCL,TEMPL
      COMMON /CCELL1/ PMVV,R,TEMPV,RFTEMP
      COMMON /CCELL6/ RT,RTRF
C
C
C  THE FOLLOWING TRANSMEMBRANE VOLUME FLOW CALCULATION TAKES
C  INTO ACCOUNT ALL COMPONENT FLUXES AS PART OF THE FLOW.
C
      SUM=0.0D0
      DO 10 K=1,SOLNP1
10    SUM=SUM+(PMVV(K)*NFLUXV(K))
      CVFUN=YLL*SUM*(RT/RTRF)
      RETURN
      END
C
C
C*********************************************************************
C
C  ROUTINE NAME   -CAFUN
C
C  PURPOSE   -THIS FUNCTION SUBPROGRAM COMPUTES THE TIME DERIVATIVE
C             OF THE DIMENSIONLESS CELL MEMBRANE SURFACE AREA.
C
C             THE EQUATION DESCRIBING THE CHANGE MUST BE WRITTEN IN
C             TERMS OF THE RELATIVE CELL VOLUME CHANGE UNLESS THE
C             CELL AREA IS ASSUMED TO STAY CONSTANT DURING THE
C             PROCESS.
C
C  USAGE   -XYZ=CAFUN(YL,YPRL)
C
C  ARGUMENTS
C
C       YL -CURRENT VALUE OF THE RELATIVE CELL VOLUME.
C     YPRL -CURRENT VALUE OF THE RELATIVE CELL VOLUME TIME
C           DERIVATIVE.
C
C
      DOUBLE PRECISION FUNCTION CAFUN(YL,YPRL)
C
C
      DOUBLE PRECISION YL,YPRL
C
C
      CAFUN=0.0D0
      RETURN
      END
C
C
C*********************************************************************
C
C  ROUTINE NAME   -CCFUN
C
C  PURPOSE   -THIS FUNCTION SUBPROGRAM EVALUATES THE SPECIES
C             DIMENSIONLESS CELL CONC. TIME DERIVATIVES. SINCE
C             THERE MIGHT BE A CERTAIN AMOUNT OF NON-OSMOTIC VOLUME
C             IN THE CELL, THE SOLUTION VOLUME IS LESS THAN THE
C             MEASURED CELL VOLUME BY AN AMOUNT OF DEAD SPACE RNOV.
C
C  USAGE   -XYZ=CCFUN(YL,YLL,YX,NCSFX,NFLUXX,YPRL)
C
C  ARGUMENTS
C
C         YL -CURRENT VALUE OF THE RELATIVE CELL VOLUME.
C        YLL -CURRENT VALUE OF THE RELATIVE CELL MEMBRANE SURFACE
C             AREA.
C         YX -CURRENT DIMENSIONLESS XTH SPECIES CELL CONCENTRATION.
C      NCSFX -XTH SPECIES NORMALIZATION FACTOR.
C     NFLUXX -CURRENT XTH SPECIES NORMALIZED FLUX.
C       YPRL -CURRENT VALUE OF THE RELATIVE CELL VOLUME TIME
C             DERIVATIVE.
C
C
      DOUBLE PRECISION FUNCTION CCFUN(YL,YLL,YX,NCSFX,NFLUXX,YPRL)
```

```
C
C
      DOUBLE PRECISION YL,YLL,YX,NCSFX,NFLUXX,YPRL,RT,RTRF,
     &                 CAREAO,VCO,RNOV
C
      COMMON /CCELL3/ CAREAO,VCO,RNOV
      COMMON /CCELL8/ RT,RTRF
C
C
      CCFUN=((YLL/(YL-RNOV))*(NFLUXX/NCSFX)*(RT/RTRF))-
     &      (YX*YPRL/(YL-RNOV))
      RETURN
      END
C
C
C**********************************************************************
C
C  ROUTINE NAME  -BUILD
C
C  PURPOSE  -THIS SUBROUTINE IS USED TO TRANSFORM ANY AM MATRIX
C            COLUMN TO A FULL MODIFIED PHENOMENOLOGICAL COEFF.
C            MATRIX (MONSAM) USED IN THE FLUX CALCULATIONS.
C
C  USAGE  -CALL BUILD(SAVGCV)
C
C  ARGUMENTS AND VARIABLES
C
C  SAVGCV -VECTOR OF THE CURRENT SOLUTE AVERAGE CONC.
C    RTRF -UNIVERSAL GAS CONSTANT MULTIPLIED BY THE REFERENCE
C          TEMPERATURE (DYNE CM GMOLE-1).
C      RT -UNIVERSAL GAS CONSTANT MULTIPLIED BY CURRENT TEMPERATURE
C          OF SYSTEM (DYNE CM GMOLE-1).
C     PHI -DIMENSIONALIZATION FACTOR USED TO GENERATE THE MODIFIED
C          FORM OF THE MACROSCOPIC PHENOMENOLOGICAL COEFFICIENTS
C          (DYNE GMOLE-1).
C  AVCONC -AVERAGE CONCENTRATION VALUE TO BE USED TO GENERATE A
C          MODIFIED COEFF.
C   LIMIT -INDEX OF THE LAST COLUMN AND LAST ROW OF MONSAM THAT
C          CONTAIN PARAMETERS TRANSFERRED FROM AM MATRIX.
C   DSQRT -FORTRAN DOUBLE-PRECISION BUILT-IN FUNCTION
C
C
      SUBROUTINE BUILD(SAVGCV)
C
C
C  DIMENSION AM(T2,T3),RESPV(T3),SAVGCV(T1),MONSAM(T1+1,T1+1),
C            PMVV(T1+1),TEMPV(T5).
C
      DIMENSION AM(14,103),RESPV(103),SAVGCV(2),MONSAM(3,3),PMVV(3),
     &          TEMPV(6)
C
      DOUBLE PRECISION AM,RESPV,SAVGCV,AVCONC,MONSAM,PMVV,R,TEMPV,
     &                 CAREAO,VCO,RNOV,PHI,RT,DSQRT,DEXP,RFTEMP,RTRF
C
      INTEGER VMAX,VMAXP1,SOLNUM,SOLNP1,IMPNUM,CONCL,TEMPL,TIND,
     &        CLIND,AMCIND,LIMIT,PCONUM,NCTIND
C
      COMMON /COPT1/ AM,RESPV
      COMMON /COPT3/ VMAX,VMAXP1,SOLNUM,SOLNP1,IMPNUM,CONCL,TEMPL
      COMMON /CCELL1/ PMVV,R,TEMPV,RFTEMP
      COMMON /CCELL3/ CAREAO,VCO,RNOV
      COMMON /CCELL5/ TIND,CLIND,AMCIND,NCTIND
      COMMON /CCELL6/ MONSAM
      COMMON /CCELL8/ RT,RTRF
C
C
C  ESTIMATE RTRF, RT AND PHI FACTORS.
C
      RTRF=R*(RFTEMP+273.15D0)
      RT=R*(TEMPV(TIND)+273.15D0)
      PHI=CAREAO*RTRF/VCO
C
C  SET ALL ELEMENTS TO 0.
C
      DO 10 I=1,SOLNP1
      DO 10 J=1,SOLNP1
10    MONSAM(I,J)=0.0D0
C
C  THEN FILL MONSAM WITH AM COLUMN PARAMETERS, I.E., OPTIMIZED
C  PARAMETERS TO BE USED IN THE FLUX CALCULATIONS. NOTE THAT EACH
C  OF THEM HAVE TO BE MULTIPLIED BY THE APPROPRIATE
C  AVERAGE CONCENTRATION TO LEAD TO THE APPROPRIATE MODIFIED
C  PHENOMENOLOGICAL COEFFICIENT.
C
      PCONUM=(SOLNUM-IMPNUM+1)*(SOLNUM-IMPNUM+2)/2
      LIMIT=SOLNUM-IMPNUM+1
      DO 20 I=1,LIMIT
      DO 20 J=1,LIMIT
      IF (I .LT. J) GO TO 20
      K=(I*(I-1)/2)+J
C
      IF ((I .EQ. 1) .AND. (J .EQ. 1)) AVCONC=1.0D0/PMVV(1)
      IF ((J .EQ. 1) .AND. (I .NE. 1)) AVCONC=DSQRT(SAVGCV(I-1)
     &                                                 /PMVV(1))
      IF ((J .NE. 1) .AND. (J .EQ. I)) AVCONC=SAVGCV(J-1)
      IF ((J .NE. 1) .AND. (J .NE. I)) AVCONC=DSQRT(SAVGCV(J-1)
     &                                              *SAVGCV(I-1))
C
      IF (TEMPL .GE. 2) GO TO 30
      MONSAM(I,J)=PHI*AM(K,AMCIND)*AVCONC
      GO TO 40
30    CONTINUE
      MONSAM(I,J)=PHI*AM(K,AMCIND)*AVCONC*
     &        DEXP(AM(PCONUM+K,AMCIND)*((1.0D0/RTRF)-(1.0D0/RT)))
40    CONTINUE
      MONSAM(J,I)=MONSAM(I,J)
20    CONTINUE
      RETURN
      END
C
C
C**********************************************************************
C
C  ROUTINE NAME  -CHECK
C
C  PURPOSE  -THIS SUBROUTINE VERIFIES IF THE THEORETICAL LIMITATIONS
C            IMPOSED ON THE ONSAGER'S PHENOMENOLOGICAL COEFFICIENTS
C            ARE RESPECTED, I.E.:
C
C                        L(I,I) .GE. 0
```

```
C                DABS(L(I,J)) .LE. DSQRT(L(I,I) * L(J,J))
C
C          IF ANY TEST FAILS THE VARIABLE CHECKF IS SET TO 1 AND
C          THE CALLING ROUTINE MUST TAKE THE APPROPRIATE ACTIONS.
C
C          THE TESTS ARE PERFORMED ON THE MODIFIED FORM OF THE
C          COEFFICIENTS (SEE SUBROUTINE BUILD).
C
C  USAGE   -CALL CHECK
C
C  ARGUMENTS AND VARIABLES
C
C     DABS,DSQRT -FORTRAN DOUBLE-PRECISION BUILT-IN FUNCTIONS.
C
C
      SUBROUTINE CHECK
C
C
C  DIMENSION MONSAM(T1+1,T1+1).
C
      DIMENSION MONSAM(3,3)
C
      DOUBLE PRECISION MONSAM,DABS,DSQRT
C
      INTEGER CHECKF,ADF,VMAX,VMAXP1,SOLNUM,SOLNP1,IMPNUM,CONCL,
     @        TEMPL
C
      COMMON /COPT3/ VMAX,VMAXP1,SOLNUM,SOLNP1,IMPNUM,CONCL,TEMPL
      COMMON /COPT5/ CHECKF,ADF
      COMMON /CCELL6/ MONSAM
C
C
C  SET FLAG TO 0.
C
      CHECKF=0
C
C  CHECK IF ALL L(I,I) ARE POSITIVE. THESE PARAMETERS ARE RELATED
C  TO THE ELEMENTS OF MONSAM MAIN DIAGONAL.
C
      DO 10 I=1,SOLNP1
      IF (.NOT.(MONSAM(I,I) .LT. 0.0D0)) GO TO 10
      CHECKF=1
      WRITE(2,2000)
2000  FORMAT(/,' ***** WARNING L(I,I) COEFF .LT. 0 *****')
C
C  STOP CHECK PROCESS IF FLAG HAS BEEN SET TO 1.
C
      GO TO 30
10    CONTINUE
C
C  CHECK IF DABS(L(I,J)) .LE. DSQRT(L(I,I)*L(J,J)).
C
      DO 20 J=1,SOLNUM
      JP1=J+1
      DO 20 I=JP1,SOLNP1
      IF (.NOT.(DABS(MONSAM(I,J)) .GT.
     @          DSQRT(MONSAM(J,J)*MONSAM(I,I))))
     @   GO TO 20
      CHECKF=1
      WRITE(2,2010)
2010  FORMAT(/,' ***** WARNING DABS(L(I,J)) .GE. DSQRT(L(I,I)',
     @          ' * L(J,J)) *****')
C
C  STOP CHECK PROCESS IF FLAG HAS BEEN SET TO 1.
C
      GO TO 30
20    CONTINUE
30    RETURN
      END
C
C
C*******************************************************************
C
C  ROUTINE NAME -OUTPUT
C
C  PURPOSE       -THIS SUBROUTINE OUTPUTS THE TABLE OF PREDICTED
C                 VS OBSERVED Y AT THE OPTIMUM AND THEIR ASSOCIATED
C                 RESIDUALS. IT ALSO GENERATES A PLOT OF EXPERIMENTAL
C                 AND PREDICTED DATA VS TIME.
C
C  USAGE         -CALL OUTPUT
C
C  VARIABLES
C
C            CALCYV -VECTOR OF PREDICTED Y VALUES.
C              RESV -VECTOR OF RESIDUAL VALUES.
C
C  PROGRAMMING NOTES
C                    IMSL ROUTINES UGETIO (I/O CONTROL) AND USPLTD
C                    (PLOTTING) ARE UTILIZED IN THIS SUBROUTINE.
C                    CONSULT IMSL LIBRARY FOR DETAILS ON THEIR USE
C                    AND THE DEFINITION OF THE VARIABLES.
C
C
      SUBROUTINE OUTPUT
C
C
C  DIMENSION XV(T4),YV(T4),WTV(T4),CALCYV(T7),RESV(T7),YM(T7,2),
C          CASEM(T6,T5),PMVV(T1+1),TEMPV(T5),CSIOV(T1),
C          CSSOLM(T6,T1).
C
      DIMENSION XV(4000),YV(4000),WTV(4000),CALCYV(120),RESV(120),
     @        YM(120,2),CASEM(20,6),PMVV(3),TEMPV(6),CSIOV(2),
     @        CSSOLM(20,2)
C
      DOUBLE PRECISION XV,YV,WTV,CALCYV,RESV,CHISQR,YM,PMVV,R,TEMPV,
     @                 CSIOV,CSSOLM,TOTSQR,RFTEMP
C
      REAL RANGE(4)
C
      INTEGER VMAX,VMAXP1,BEST,WORST,NEWPNT,TOTCNT,CHECKF,ADF,CASEM,
     @        SOLNUM,SOLNP1,IMPNUM,CONCL,TEMPL,TIND,CLIND,AMCIND,
     @        NCOUNT,NCASES,NCTIND
C
      INTEGER IY,N,M,INC,IOPT,IMAG4(5151),IER,ITITLE(144),ICHAR(10)
C
      DATA ICHAR(1)/'E'/,ICHAR(2)/'P'/,RANGE/4*0.0/
      DATA ITITLE/'P','L','O','T',' ','O','F',' ','V','O','L','U',
     @          'M','E',' ','C','H','A','N','G','E',' ','O','F',
     @          ' ','S','T','U','D','I','E','D',' ','C','E','L',
     @          'L','S',':',' ',' ','E','X','P','E','R','I','M','E',
     @          'N','T','A','L',' ','(','E',')',' ',' ',' ','P','R',
     @          'E','D','I','C','T','E','D',' ','(','P',')',' ',' ',
     &          12*' ','T','I','M','E',' ','(','S','E','C',')',
     @          ' ',13*' ',4*' ','R','E','L','A','T','I','V','E',
     @          ' ','C','E','L','L',' ','V','O','L','U','M','E',
     @          ' ',' ','V','/','V','O',8*' '/
C
      COMMON /COPT2/ YV,WTV,XV,CASEM
      COMMON /COPT3/ VMAX,VMAXP1,SOLNUM,SOLNP1,IMPNUM,CONCL,TEMPL
      COMMON /COPT4/ BEST,WORST,NEWPNT,TOTCNT
      COMMON /COPT5/ CHECKF,ADF
      COMMON /CCELL1/ PMVV,R,TEMPV,RFTEMP
      COMMON /CCELL2/ CSIOV,CSSOLM
      COMMON /CCELL5/ TIND,CLIND,AMCIND,NCTIND
C
C
      WRITE(2,2060)
2060  FORMAT(//,' ',100('_'),//,' )))))) TABLES OF PREDICTED',
     @        ' VS OBSERVED RELATIVE CELL VOLUMES.',//)
C
      NCOUNT=1
      TOTSQR=0.0D0
      DO 60 J=1,TEMPL
      DO 60 I=1,CONCL
      IF (CASEM(I,J) .EQ. 0) GO TO 60
C
      WRITE(2,2030) TEMPV(J)
2030  FORMAT(///,' TEMPERATURE OF SYSTEM: ',F5.1,//,' SOLUTE',
     @        ' CONCENTRATIONS IN OSMOTICUM.',/,'  SOLUTE INDEX',
     @        '      CONCENTRATION',/)
      DO 70 K=1,SOLNUM
      WRITE(2,2040) K,CSSOLM(I,K)
2040  FORMAT(7X,I1,11X,1PD10.3)
70    CONTINUE
C
      WRITE(2,2000)
2000  FORMAT(///,3X,'TIME',5X,'OBSERVED',8X,'PREDICTED',8X,
     @             'RESIDUALS',8X,'WEIGHTS',/)
C
      TIND=J
      CLIND=I
      AMCIND=TOTCNT+1
      NCTIND=NCOUNT
      CALL CELLCH(CALCYV)
C
C  CHECK IF CHECKF AND/OR ADF HAVE BEEN SET TO 1. IF SO END
C  PROCEDURE AND GO BACK TO CALLING ROUTINE.
C
      IF ((CHECKF .EQ. 1) .OR. (ADF .EQ. 1)) GO TO 20
C
      CHISQR=0.0D0
      NCASES=CASEM(I,J)
      DO 10 K=1,NCASES
C
C  CALCULATE THE CHI-SQUARE AT THE OPTIMUM FROM THE GENERATED
C  RESIDUALS.
C
      RESV(K)=YV(NCOUNT)-CALCYV(K)
      CHISQR=CHISQR+(WTV(NCOUNT)*(RESV(K)**2))
C
      YM(K,1)=YV(NCOUNT)
      YM(K,2)=CALCYV(K)
      WRITE(2,2010) XV(NCOUNT),YV(NCOUNT),CALCYV(K),RESV(K),
     @               WTV(NCOUNT)
2010  FORMAT(' ',F6.2,6X,F6.4,11X,F6.4,10X,1PD10.3,6X,D10.3)
      NCOUNT=NCOUNT+1
10    CONTINUE
C
      WRITE(2,2020) CHISQR
2020  FORMAT(//,' CALCULATED CHI-SQUARE AT OPTIMUM: ',1PD10.3,///)
      TOTSQR=TOTSQR+CHISQR
C
C  INITIALIZE PLOT ROUTINE PARAMETERS
C
      CALL UGETIO (3,1,2)
      INC=1
      N=CASEM(I,J)
      M=2
      IOPT=1
      IY=120
      CALL USPLTD(XV,YM,IY,N,M,INC,ITITLE,RANGE,ICHAR,IOPT,IMAG4,
     &            IER)
60    CONTINUE
      WRITE(2,2050) TOTSQR
2050  FORMAT(//,' TOTAL SUM OF SQUARES FOR ALL SETS: ',1PD10.3,///)
20    RETURN
      END
```

13.2.3 The "optimization" routines

This unit reassembles all the subroutines pertaining to the Simplex search method. Since this algorithm is described in detail in Appendix 1, the reader is referred to that chapter for a complete description of the routines.

```
C*********************************************************************
C
C  THIS FILE CONTAINS THE ROUTINES REFERRED TO AS "OPTIMIZATION"
C  ROUTINES IN PROGRAM MCPERM. THEY ARE ESSENTIALLY USED TO CARRY
C  THE SIMPLEX OPTIMIZATION SEARCH OF THE BEST MODIFIED PHENOMENOLO-
C  GICAL COEFFICIENTS (LEAST-SQUARES SENSE) DESCRIBING THE
C  PERMEABILITY CHARACTERISTICS OF THE CELLS UNDER STUDY.
C
C  THE ROUTINES ARE:
C
C        MEAS  -COMPUTES THE LEAST-SQUARES VALUE CORRESPONDING TO
C               ANY GIVEN SET OF OPTIMIZED PARAMETERS.
C      PDELTA  -EVALUATES THE STEPS NEEDED TO GENERATE THE STARTING
C               SIMPLEX.
C       SETUP  -GENERATES THE STARTING SIMPLEX.
C        MOVE  -USED TO MOVE COLUMNS OF AM MATRIX DURING SIMPLEX
C               SEARCH.
C      GENNEW  -GENERATES NEW VERTICES FOR THE SIMPLEX.
C      MODSPX  -SIMPLEX OPTIMIZATION PROCEDURE (MAIN DRIVER).
C      EXPAND  -EXPANDS THE FINAL SIMPLEX AROUND THE CENTROID.
C      QUADOP  -USED TO ESTIMATE THE INFORMATION MATRIX IN THE
C               NEIGHBOURHOOD OF THE MINIMUM. PERFORMS A QUADRATIC
C               APPROXIMATION TO GET A BETTER ESTIMATE OF THE MINIMUM
C      DETERM  -ANALYSES THE CHARACTER OF THE STATIONARY POINT
C               CALCULATED IN QUADOP.
C       STERR  -ESTIMATES THE STANDARD ERRORS OF THE OPTIMIZED
C               PARAMETERS.
C        ACCU  -ESTIMATES THE GLOBAL TRUNCATION ERRORS OF THE
C               PREDICTED RELATIVE CELL VOLUME VALUES (COMPUTED BY
C               ROUTINE CELLCH).
C
C*********************************************************************
C
C        **********   LATEST VERSION MARCH 28, 1985   **********
C                          BY CHRISTIAN TOUPIN.
C
C*********************************************************************
C                      OPTIMIZATION SUBROUTINES.
C*********************************************************************
C
C  ROUTINE NAME   -MEAS
C
C  PURPOSE        -THIS ROUTINE USES THE VALUES OF THE PARAMETERS
C                  IN THE M-TH COLUMN OF AM TO CALCULATE A NEW
C                  RESPV(M). THE RESPONSE IS SIMPLY THE WEIGHTED
C                  CHI-SQUARE VALUE OBTAINED BY PLUGGING THE ELEMENTS
C                  OF THE M-TH COLUMN OF AM INTO THE DIFF. EQUATIONS
C                  GETTING THE PREDICTED Y (CALCYV) WHICH ARE THEN
C                  COMPARED WITH THE OBSERVED ONES. THE SQUARE OF THE
C                  RESULTING RESIDUALS ONCE WEIGHTED ARE ADDED TO
C                  CHISQR, WHICH BECOMES THE RESPONSE RESPV(M) WHEN
C                  DONE.
C
C  USAGE          -CALL MEAS(M)
C
C  ARGUMENTS AND VARIABLES
C
C                              M -AM COLUMN INDEX OF PARAMETER VALUES TO
C                                 BE USED IN THE CALCULATION OF THE
C                                 PREDICTED Y.
C                         NCOUNT -POSITION COUNTER.
C                           TIND -TEMPERATURE INDEX INDICATING THE
C                                 CURRENT TEMPERATURE OF THE SYSTEM.
C                          CLIND -CONCENTRATION LEVEL INDEX INDICATING
C                                 THE CURRENT OSMOTICUM CONCENTRATION
C                         AMCIND -M INDEX TRANSMITTED THROUGH COMMON
C                                 /CCELLS/.
C                         NCTIND -INITIAL VALUE OF POSITION COUNTER
C                                 NCOUNT.
C                         CALCYV -VECTOR OF PREDICTED Y VALUES.
C                         CHISQR -CHI-SQUARE VALUE CORRESPONDING TO THIS
C                                 SET OF PREDICTED Y.
C
C
      SUBROUTINE MEAS(M)
C
C
C  DIMENSION AM(T2,T3),RESPV(T3),YV(T4),WTV(T4),XV(T4),CASEM(T5,T6),
C           CALCYV(T7).
C
      DIMENSION AM(14,103),RESPV(103),YV(4000),WTV(4000),XV(4000),
     &          CASEM(20,8),CALCYV(120)
C
      DOUBLE PRECISION AM,RESPV,YV,WTV,XV,CALCYV,CHISQR
C
      INTEGER CASEM,VMAX,VMAXP1,SOLNUM,SOLNP1,IMPNUM,CHECKF,M,
     &        ADF,CONCL,TEMPL,TIND,CLIND,AMCIND,NCOUNT,NCASES,NCTIND
C
      COMMON /COPT1/ AM,RESPV
      COMMON /COPT2/ YV,WTV,XV,CASEM
      COMMON /COPT3/ VMAX,VMAXP1,SOLNUM,SOLNP1,IMPNUM,CONCL,TEMPL
      COMMON /COPT5/ CHECKF,ADF
      COMMON /CCELLS/ TIND,CLIND,AMCIND,NCTIND
C
C
C  SET CHISQR TO 0.
C
      CHISQR=0.0D0
      NCOUNT=1
      DO 10 J=1,TEMPL
      DO 10 I=1,CONCL
      IF (CASEM(I,J) .EQ. 0) GO TO 10
      TIND=J
      CLIND=I
      AMCIND=M
      NCTIND=NCOUNT
C
C  GET THE PREDICTED Y VALUES.
C
      CALL CELLCH(CALCYV)
C
C  IF CHECKF AND/OR ADF HAVE BEEN SET TO 1, END PROCEDURE AND GO
C  BACK TO CALLING ROUTINE.
C
      IF ((CHECKF .EQ. 1) .OR. (ADF .EQ. 1)) GO TO 20
C
C  OTHERWISE CALCULATE THE CHI-SQUARE VALUE.
C
      NCASES=CASEM(I,J)
      DO 10 K=1,NCASES
      CHISQR=CHISQR+(WTV(NCOUNT)*((YY(NCOUNT)-CALCYY(K))**2))
      NCOUNT=NCOUNT+1
10    CONTINUE
C
C  RETURN CHI-SQUARE VALUE AS MTH ELEMENT OF RESPV.
C
      RESPV(M)=CHISQR
20    CONTINUE
      RETURN
      END
C
C
C***********************************************************************
C
C  ROUTINE NAME   -PDELTA
C
C  PURPOSE        -THIS SUBR. CALCULATES THE STEPS NEEDED TO SET UP
C                  THE INITIAL SIMPLEX. IT CALCULATES AS STEP (FOR
C                  EACH PARAMETER) 10% OF THE PARAMETER INITIAL GUESS
C                  VALUE.
C
C  USAGE          -CALL PDELTA(DELTAV)
C
C  ARGUMENTS
C
C            DELTAV -VECTOR OF THE STEPS NEEDED TO SET UP THE
C                    INITIAL SIMPLEX. USED IN SUBR. SETUP.
C
C
      SUBROUTINE PDELTA(DELTAV)
C
C
C  DIMENSION AM(T2,T3),RESPV(T3),DELTAV(T2).
C
      DIMENSION AM(14,103),RESPV(103),DELTAV(14)
C
      DOUBLE PRECISION AM,RESPV,DELTAV
C
      INTEGER VMAX,VMAXP1,SOLNUM,SOLNP1,IMPNUM,CONCL,TEMPL
C
      COMMON /COPT1/ AM,RESPV
      COMMON /COPT3/ VMAX,VMAXP1,SOLNUM,SOLNP1,IMPNUM,CONCL,TEMPL
C
C
      DO 10 I=1,VMAX
10    DELTAV(I)=0.10D0*AM(I,1)
      RETURN
      END
C
C
C***********************************************************************
C
C  ROUTINE NAME   -SETUP
C
C  PURPOSE        -MAKES THE INITIAL VMAXP1 MEASUREMENTS TO GENERATE
C                  THE STARTING SIMPLEX. A SIMPLEX ALWAYS CONTAINS
C                  ONE MORE VERTEX THAN THE TOTAL NUMBER OF PARAM.
C                  TO OPTIMIZE, I.E., VMAXP1. THIS ROUTINE USES THE
C                  DELTAV STEPS TO CALCULATE THE VMAX OTHER VERTICES
C                  NEEDED.
C
C  USAGE          -CALL SETUP(DELTAV)
C
C  ARGUMENTS
C
C             DELTAV -VECTOR OF THE STEPS NEEDED TO SET UP THE
C                     INITIAL SIMPLEX. GENERATED IN SUBR. PDELTA.
C
C
      SUBROUTINE SETUP(DELTAV)
C
C
C  DIMENSION AM(T2,T3),RESPV(T3),DELTAV(T2)
C
      DIMENSION AM(14,103),RESPV(103),DELTAV(14)
C
      DOUBLE PRECISION AM,RESPV,DELTAV
C
      INTEGER VMAX,VMAXP1,SOLNUM,SOLNP1,IMPNUM,CONCL,TEMPL,CHECKF,
     *        ADF
C
      COMMON /COPT1/ AM,RESPV
      COMMON /COPT3/ VMAX,VMAXP1,SOLNUM,SOLNP1,IMPNUM,CONCL,TEMPL
      COMMON /COPT5/ CHECKF,ADF
C
C
      VMAXP1=VMAX+1
      DO 10 J=1,VMAXP1
      CALL MEAS(J)
C
C  IF CHECKF AND/OR ADF HAVE BEEN SET TO 1, END PROCEDURE AND GO
C  BACK TO CALLING ROUTINE.
C
      IF ((CHECKF .EQ. 1) .OR. (ADF .EQ. 1)) GO TO 20
C
C  OTHERWISE CALCULATE THE NEXT VERTEX PARAMETERS FROM THE VALUES
C  OF DELTAV.
C
      DO 10 I=1,VMAX
      IF (I .EQ. J) AM(I,J+1)=AM(I,1)+DELTAV(I)
      IF (I .NE. J) AM(I,J+1)=AM(I,1)
10    CONTINUE
20    CONTINUE
      RETURN
      END
C
C
C***********************************************************************
C
C  ROUTINE NAME   -MOVE
C
C  PURPOSE        -PUTS THE VARIABLES OF THE M-TH COLUMN OF AM IN THE
C                  L-TH COLUMN. MOVES THE ELEMENTS IN RESPV
C                  ACCORDINGLY.
C
C  USAGE          -CALL MOVE(L,M)
C
C  ARGUMENTS
C
```

```
C               L -INDEX OF THE L-TH COLUMN OF AM.
C               M -INDEX OF THE M-TH COLUMN OF AM.
C
C
      SUBROUTINE MOVE(L,M)
C
C
C  DIMENSION AM(T2,T3),RESPV(T3).
C
      DIMENSION AM(14,103),RESPV(103)
C
      DOUBLE PRECISION AM,RESPV
C
      INTEGER VMAX,VMAXP1,SOLNUM,SOLNP1,IMPNUM,CONCL,TEMPL,L,M
C
      COMMON /COPT1/ AM,RESPV
      COMMON /COPT3/ VMAX,VMAXP1,SOLNUM,SOLNP1,IMPNUM,CONCL,TEMPL
C
C
      DO 10 I=1,VMAX
10    AM(I,L)=AM(I,M)
      RESPV(L)=RESPV(M)
      RETURN
      END
C
C
C**************************************************************
C
C  ROUTINE NAME   -GENNEW
C
C  PURPOSE        -GENERATES NEW VERTICES FOR THE SIMPLEX BY
C                  MULTIPLYING THE DIFFERENCE BETWEEN THE WORST AND
C                  THE CENTROID BY THE FACTOR F, AND THEN MEASURING
C                  THE NEW VALUE. KEEPS THE NEW VALUE ONLY IF BETTER
C                  THAN THE ORIGINAL ONE.
C
C  USAGE          -CALL GENNEW(M,F)
C
C  ARGUMENTS
C
C               M -INDEX OF AM COLUMN WHERE CALCULATED VERTEX IS
C                  TO BE SENT.
C               F -REFLECTION, EXPANSION OR CONTRACTION FACTOR
C                  USED TO GENERATE NEW VERTEX.
C          CENTRV -CENTROID PARAMETER VALUES, SENT FROM MODSPX
C                  THROUGH COMMON /COPT6/
C           HOLDV -BUFFER VECTOR USED TO SAVE THE PARAMETER VALUES
C                  IN CASE OF FAILURE OF CHECK TEST.
C            HOLD -BUFFER SCALAR USED TO SAVE THE RESPONSE VALUE
C           DMIN1 -FORTRAN DOUBLE-PRECISION BUILT-IN FUNCTION.
C
C
      SUBROUTINE GENNEW(M,F)
C
C
C  DIMENSION AM(T2,T3),RESPV(T3),CENTRV(T2),HOLDV(T2).
C
      DIMENSION AM(14,103),RESPV(103),CENTRV(14),HOLDV(14)
C
      DOUBLE PRECISION AM,RESPV,CENTRV,F,HOLDV,HOLD,DMIN1
C
      INTEGER VMAX,VMAXP1,WORST,NEWPNT,TOTCNT,CHECKF,M,
     @        ADF,SOLNUM,SOLNP1,IMPNUM,BEST,CONCL,TEMPL
C
      COMMON /COPT1/ AM,RESPV
      COMMON /COPT3/ VMAX,VMAXP1,SOLNUM,SOLNP1,IMPNUM,CONCL,TEMPL
      COMMON /COPT4/ BEST,WORST,NEWPNT,TOTCNT
      COMMON /COPT5/ CHECKF,ADF
      COMMON /COPT6/ CENTRV
C
C
      DO 10 I=1,VMAX
10    AM(I,M)=CENTRV(I)+(F*(CENTRV(I)-AM(I,WORST)))
      CALL MEAS(M)
C
C  CHECK IF THE NEWLY GENERATED VERTEX RESPECTS THE LIMITATIONS
C  IMPOSED ON THE PARAMETERS. IF NOT, THE RESPONSE IS SET TO A
C  VERY UNDESIRABLE VALUE IN ORDER TO FORCE THE SIMPLEX TO MOVE
C  IN ANOTHER DIRECTION. IF A MALFUNCTION OF SUBR. ODE (IN SUBR.
C  CELLCH) HAS BEEN DETECTED (ADF .EQ. 1), END PROCEDURE AND GO
C  BACK TO CALLING ROUTINE.
C
      IF (CHECKF .EQ. 1) RESPV(M)=1.0D36
      IF (ADF .EQ. 1) GO TO 20
C
C  REPLACE WORST VALUE WITH THE NEW ONE IF THE RESPONSE IS BETTER
C  AND IF THIS IS NOT THE INITIAL MEASUREMENT WITH THE FACTOR OF 1.
C  IF A FAILED CONTRACTION OCCURS, SHRINK THE ENTIRE SIMPLEX
C  HALFWAY TOWARD THE BEST VERTEX.
C
C  REFLECTION PROCESS.
C
      IF (F .EQ. 1.0D0) GO TO 20
C
C  EXPANSION PROCESS.
C
      IF (F .NE. 2.5D0) GO TO 30
      IF (RESPV(M) .LT. RESPV(NEWPNT)) GO TO 40
      CALL MOVE(WORST,NEWPNT)
      GO TO 20
40    CONTINUE
      CALL MOVE(WORST,M)
      GO TO 20
30    CONTINUE
C
C  CONTRACTION PROCESS.
C
      IF (RESPV(M) .LT. DMIN1(RESPV(WORST),RESPV(NEWPNT))) GO TO 50
C
C  ON FAILURE OF CONTRACTION PROCESS, SHRINK THE ENTIRE SIMPLEX
C  HALFWAY TOWARD THE BEST VERTEX. IF THE VALUES OF THE PARAMETERS
C  FOR A GIVEN VERTEX FALL OUT OF THEIR DOMAIN OF VALIDITY DURING
C  THE OPERATION, STOP THE SHRINKAGE FOR THIS VERTEX AND RESTORE
C  THE INITIAL POINT.
C
      WRITE(2,2000)
2000  FORMAT(/,' **** SHRINKAGE OF SIMPLEX PERFORMED. ****')
      DO 60 J=1,VMAXP1
C
C  SKIP OPERATION FOR BEST POINT.
```

```
C
      IF (J .EQ. BEST) GO TO 60
C
C  SAVE VERTEX PARAMETER VALUES AND RESPONSE.
C
      HOLD=RESPV(J)
      DO 70 I=1,VMAX
      HOLDV(I)=AM(I,J)
70    CONTINUE
      DO 80 I=1,VMAX
      AM(I,J)=AM(I,BEST)-0.5D0*(AM(I,BEST)-AM(I,J))
80    CONTINUE
C
C  GET RESPONSE AT NEW VERTEX.
C
      CALL MEAS(J)
C
C  CHECK IF NEW VERTEX PARAMETER VALUES ARE VALID. IF NOT RESTORE
C  INITIAL POINT AND MOVE TO THE NEXT VERTEX. IF ADF HAS BEEN SET
C  TO 1, END PROCEDURE AND GO BACK TO CALLING ROUTINE.
C
      IF (ADF .EQ. 1) GO TO 20
      IF (CHECKF .NE. 1) GO TO 60
C
C  RESTORE INITIAL POINT VALUES.
C
      RESPV(J)=HOLD
      DO 90 I=1,VMAX
      AM(I,J)=HOLDV(I)
90    CONTINUE
      CHECKF=0
60    CONTINUE
      GO TO 20
50    CONTINUE
      CALL MOVE(WORST,M)
20    CONTINUE
      RETURN
      END
C
C
C***********************************************************************
C
C  ROUTINE NAME   -MODSPX
C
C  PURPOSE        -SIMPLEX OPTIMIZATION PROCEDURE, OPERATES ON VMAX
C                  DIFF. PARAMETERS IN A MAT. AM(PARAMETERS,VERTICES)
C                  WHERE THERE MUST ALWAYS BE ONE MORE VERTEX THAN
C                  THE TOTAL NUMBER OF PARAMETERS TO BE VARIED. SINCE
C                  WE ARE MINIMIZING THE LEAST-SQUARES RESPONSE, BEST
C                  IS THE SMALLEST RESPONSE AND WORST THE LARGEST.
C
C                  THE GENERATED VERTICES DURING THE PROCESS ARE
C                  CHECKED TO ENSURE THAT THE PARAMETERS RESPECT
C                  THE THEORETICAL LIMITATIONS IMPOSED ON THEM. IN
C                  CASE OF FAILURE THE SIMPLEX IS FORCED TO STAY
C                  IN THE ACCEPTABLE DOMAIN OF COEFFICIENT VALUES.
C
C  USAGE          -CALL MODSPX(TOLSPX,TOLAVE,TESTSF,ITEMAX,ITEFL)
C
C  ARGUMENTS AND VARIABLES
C
C               TOLSPX,TOLAVE -TOLERANCES BELOW WHICH SPXT AND AVERT
C                              MUST FALL. USED AS CRITERIA FOR
C                              HALTING A SIMPLEX SEARCH.
C                      TESTSF -FLAG TO INDICATE IF LISTING OF
C                              VARIABLES AVERT AND SPXT IS DESIRED
C                              (TESTSF=1).
C                      ITEMAX -MAXIMUM NUMBER OF ITERATIONS ALLOWED
C                              TO FIND AN OPTIMUM IN A GIVEN SIMPLEX
C                              SEARCH
C                       ITEFL -FLAG TO INDICATE IF THE MAXIMUM
C                              NUMBER OF ITERATIONS ALLOWED IN A
C                              SIMPLEX SEARCH HAS BEEN REACHED. IN
C                              THAT CASE ITEFL IS SET TO 1.
C   BEST,WORST,NXBEST,WORST2 -POSITION INDEXES. BEST IS THE INDEX
C                              IN RESPV AND AM (COLUMN #) OF THE
C                              VERTEX WITH THE SMALLEST CHI-SQUARE
C                              VAL., WORST THE INDEX OF THE LARGEST.
C                              NXBEST IS THE SECOND BEST AND WORST2
C                              THE SECOND WORST.
C                 NEWPNT,NEW2 -POSITION INDEXES. NEWPNT IS THE INDEX
C                              IN RESPV AND AM (COLUMN #) OF THE
C                              NEWEST MEASURED VERTEX. NEW2 IS THE
C                              INDEX OF THE SECOND VERTEX GENERATED
C                              IN RESPONSE TO THE VALUE OF THE
C                              CHI-SQUARE AT NEWPNT.
C                        ITER -ITERATION COUNTER. ITS VALUE SHOWS
C                              THE NUMBER OF SIMPLICES CALCULATED TO
C                              REACH AN OPTIMUM FOR ONE COMPLETE
C                              SEARCH PROCESS.
C                      CENTRV -VECTOR OF THE CENTROID PARAMETER VAL.
C                              SENT TO SUBR GENNEW BY COMMON /COPT6/.
C                  AVERT,SPXT -TEST VAL. USED AS CRITERIA FOR HALTING
C                              THE SIMPLEX SEARCH PROCEDURE. THE VAL.
C                              MUST BE .LE. TOLAVE AND TOLSPX.
C                     MAX,MIN -MAXIMUM AND MINIMUM RESPONSE MEASURED
C                              IN THE SIMPLEX.
C                   MAX2,MIN2 -SECOND WORST AND SECOND BEST RESPONSE
C                              MEASURE IN THE SIMPLEX.
C                         SUM -SUM OF THE SQUARES OF THE RESIDUALS OF
C                              THE SIMPLEX POPULATION OF RESPONSES.
C                              USED TO CALCULATE SPXT (SEE ROUTINE
C                              FOR DETAILS).
C                      RESPAV -AVERAGE RESPONSE OF THE STUDIED
C                              SIMPLEX (SEE ROUTINE FOR DETAILS).
C                      OLDAVE -AVERAGE RESPONSE OF THE SIMPLEX BEFORE
C                              THE CALCULATION OF A NEW VERTEX, I.E.,
C                              BEFORE A NEW MOVE TOWARD THE SOLUTION.
C                      NEWAVE -AVERAGE RESPONSE OF THE SIMPLEX AFTER
C                              THE MOVE HAS BEEN DONE. OLDAVE AND
C                              NEWAVE ARE USED TO CALCULATE AVERT.
C                  DSQRT,DABS -FORTRAN DOUBLE-PRECISION BUILT-IN
C                              FUNCTIONS.
C
C
      SUBROUTINE MODSPX(TOLSPX,TOLAVE,TESTSF,ITEMAX,ITEFL)
C
C
C  DIMENSION AM(T2,T3),RESPV(T3),CENTRV(T2).
C
```

```
      DIMENSION AM(14,103),RESPV(103),CENTRV(14)
C
      DOUBLE PRECISION AM,RESPV,CENTRV,AVERT,SPXT,MAX,MIN,MAX2,
     @      MIN2,SUM,RESPAV,OLDAVE,NEWAVE,TOLSPX,TOLAVE,DSQRT,DABS
C
      INTEGER VMAX,VMAXP1,BEST,WORST,NXBEST,WORST2,NEWPNT,NEW2,
     @        TOTCNT,TESTSF,CHECKF,ADF,ITER,SOLNUM,SOLNP1,IMPNUM,
     @        CONCL,TEMPL,ITEMAX,ITEFL
C
      COMMON /COPT1/ AM,RESPV
      COMMON /COPT3/ VMAX,VMAXP1,SOLNUM,SOLNP1,IMPNUM,CONCL,TEMPL
      COMMON /COPT4/ BEST,WORST,NEWPNT,TOTCNT
      COMMON /COPT5/ CHECKF,ADF
      COMMON /COPT6/ CENTRV
C
C
C   THIS IS THE INDEX OF THE NEWEST MEASURED VERTEX.
C
      NEWPNT=0
C
C   NUMBER OF VERTICES NEEDED ALTOGETHER.
C
      VMAXP1=VMAX+1
C
C   EXTRA COLJMN IN AM FOR NEW VERTEX.
C
      NEWPNT=VMAX+2
C
C   EXTRA COLUMN IN AM FOR A SECOND NEW VERTEX.
C
      NEW2=VMAX+3
C
C   CALCULATE THE AVERAGE RESPONSE OF THE INITIAL SIMPLEX
C
      RESPAV=0.0D0
      DO 10 K=1,VMAXP1
10    RESPAV=RESPAV+RESPV(K)
      RESPAV=RESPAV/VMAXP1
      OLDAVE=RESPAV
      WRITE(2,2000)
2000  FORMAT(//,' >>>>>>>  SIMPLEX OPTIMIZATION IN PROGRESS ',//)
      ITER=0
180   CONTINUE
C
C   FIND THE MAXIMUM AND MINIMUM RESPONSE MEASURED
C
      MAX=0 0D0
      MIN=1.0D38
      DO 20 K=1,VMAXP1
      IF (.NOT.(RESPV(K) .GT. MAX)) GO TO 30
      MAX=RESPV(K)
      WORST=K
30    CONTINUE
      IF (.NOT.(RESPV(K) .LT. MIN)) GO TO 20
      MIN=RESPV(K)
      BEST=K
20    CONTINUE
C
C   FIND 2ND BEST AND 2ND WORST, TOO.
C
      MAX2=0.0D0
      MIN2=1.0D38
      DO 40 K=1,VMAXP1
      IF (.NOT.((RESPV(K) .GT. MAX2) .AND. (RESPV(K) .LT. MAX)))
     @ GO TO 50
      MAX2=RESPV(K)
      WORST2=K
50    CONTINUE
      IF (.NOT.((RESPV(K) .LT. MIN2) .AND. (RESPV(K) .GT. MIN)))
     @ GO TO 40
      MIN2=RESPV(K)
      NXBEST=K
40    CONTINUE
C
C   CALCULATE CENTROID OF ALL MEASUREMENTS EXCEPT WORST. ZERO OUT
C   CENTROID ELEMENTS FIRST.
C
      DO 60 K=1,VMAX
60    CENTRV(K)=0.0D0
      DO 70 I=1,VMAX
      DO 70 J=1,VMAXP1
70    IF (J .NE. WORST) CENTRV(I)=CENTRV(I)+AM(I,J)
C
C   DIVIDE CENTROID DOWN BY VMAX.
C
      DO 80 K=1,VMAX
80    CENTRV(K)=CENTRV(K)/VMAX
C
C   MEASURE THE RESPONSE AT THE POINT REFLECTED AWAY FROM WORST.
C   MEASURE NEW POINT NORMALLY.
C
      CALL GENNEW(NEWPNT,1.0D0)
C
C   CHECK FOR MALFUNCTION OF SUBR. ODE (IN SUBR. CELLCH). IF SO,
C   END PROCEDURE AND GO BACK TO CALLING ROUTINE.
C
      IF (ADF .EQ. 1) GO TO 170
C
C   IF THIS ONE IS BETTER THAN PREVIOUS BEST, THEN EXPAND IN THIS
C   DIRECTION.
C
      IF (RESPV(NEWPNT) .LT. RESPV(BEST)) GO TO 90
C
C   IF THIS ONE IS WORSE THAN THE PREVIOUS WORST, MEASURE POINT
C   HALFWAY BETWEEN WORST AND CENTROID.
C
      IF (RESPV(NEWPNT) .GE. RESPV(WORST)) GO TO 100
C
C   IF NEWEST RESPONSE IS WORSE THAN THE NEXT-TO-THE-WORST BUT BETTER
C   THAN WORST, MEASURE RESPONSE HALFWAY BETWEEN CENTROID AND NEWEST
C   POINT.
C
      IF ((RESPV(NEWPNT) .GT. RESPV(WORST2)) .AND. (RESPV(NEWPNT)
     @ .LT. RESPV(WORST))) GO TO 110
C
C   IF NONE OF THE ABOVE, KEEP THE NEW POINT AS BEST.
C
      CALL MOVE(WORST,NEWPNT)
      GO TO 120
90    CONTINUE
```

```
      CALL GENNEW(NEW2,2.5D0)
C
C  CHECK FOR MALFUNCTION OF SUBR. ODE (IN SUBR. CELLCH). IF SO,
C  END PROCEDURE AND GO BACK TO CALLING ROUTINE.
C
      IF (ADF .EQ. 1) GO TO 170
      GO TO 120
100   CONTINUE
      CALL GENNEW(NEW2,-0.25D0)
C
C  CHECK FOR MALFUNCTION OF SUBR. ODE (IN SUBR. CELLCH). IF SO,
C  END PROCEDURE AND GO BACK TO CALLING ROUTINE.
C
      IF (ADF .EQ. 1) GO TO 170
      GO TO 120
110   CONTINUE
      CALL GENNEW(NEW2,0.25D0)
C
C  CHECK FOR MALFUNCTION OF SUBR. ODE (IN SUBR. CELLCH). IF SO,
C  END PROCEDURE AND GO BACK TO CALLING ROUTINE.
C
      IF (ADF .EQ. 1) GO TO 170
120   CONTINUE
C
C  INCREMENT SIMPLEX ITERATION COUNTER.
C
      ITER=ITER+1
C
C  CALCULATE THE AVERAGE RESPONSE FOR THE UPDATED SIMPLEX.
C
      RESPAV=0.0D0
      DO 130 K=1,VMAXP1
130   RESPAV=RESPAV+RESPV(K)
      RESPAV=RESPAV/VMAXP1
      NEWAVE=RESPAV
C
C  CALCULATE THE RELATIVE DIFFERENCE BETWEEN THE AVERAGE RESPONSE
C  OF THE PREVIOUS SIMPLEX AND THE AVERAGE RESPONSE OF THE UPDATED
C  ONE (AVERT).
C
C  CALCULATE THE STANDARD DEVIATION OF THIS UPDATED POPULATION OF
C  RESPONSES AND GET THE COEFFICIENT OF VARIATION (SPXT).
C
      SUM=0.0D0
      DO 140 K=1,VMAXP1
140   SUM=SUM+((RESPV(K)-RESPAV)**2)
      SPXT=(DSQRT(SUM/VMAX))/RESPAV
      AVERT=DABS((OLDAVE-NEWAVE)/NEWAVE)
C
      WRITE(2,2070) ITER, RESPV(BEST)
2070  FORMAT(/,'   ITERATION NUMBER: ',I5,/,
     &       '   CHI-SQUARE VALUE AT CURRENT BEST VERTEX: ',
     &       1PD10.3,/,'   PARAMETER VALUES: ',/)
      DO 145 I=1,VMAX
      WRITE(2,2080) I, AM(I,BEST)
2080  FORMAT(6X,'P(',I2,')',6X,1PD11.4)
145   CONTINUE
C
C  THE USER BY SETTING THE VAR. TESTSF TO 1 WILL HAVE THE LISTING
C  OF THE ABOVE TEST VARIABLES.
C
      IF (TESTSF .NE. 1) GO TO 150
      WRITE(2,2010) OLDAVE,NEWAVE,AVERT
2010  FORMAT(/,' OLDAVE:',1PD10.3,' NEWAVE:',D10.3,' AVERT:',D10.3)
      WRITE(2,2020) RESPAV,SUM,SPXT
2020  FORMAT(' RESPAV:',1PD10.3,' SUM:    ',D10.3,' SPXT: ',D10.3)
150   CONTINUE
C
C  TERMINATE THE PROCEDURE IF THE COEFFICIENT OF VARIATION AND THE
C  DIFFERENCE BETWEEN OLD AND NEW AVERAGE VALUES ARE .LE. TOLSPX
C  AND TOLAVE RESPECTIVELY OR IF THE MAXIMUM NUMBER OF ITERATIONS
C  HAS BEEN REACHED.
C
      IF (ITER .LT. ITEMAX) GO TO 152
      ITEFL=1
      GO TO 170
152   CONTINUE
      OLDAVE=NEWAVE
      IF (.NOT.((SPXT .LE. TOLSPX) .AND. (AVERT .LE. TOLAVE)))
     &   GO TO 160
      WRITE(2,2030)
2030  FORMAT(//,'   ***** OPTIMUM VERTEX FOUND. *****',/)
      DO 155 I=1,VMAX
      WRITE(2,2040) I, AM(I,BEST)
2040  FORMAT(6X,'P(',I2,')',6X,1PD11.4)
155   CONTINUE
      WRITE(2,2050) RESPV(BEST)
2050  FORMAT(/,' CHI-SQUARE VALUE AT OPTIMUM VERTEX: ',1PD10.3)
      WRITE(2,2060) ITER
2060  FORMAT(/,6X,I3,' ITERATION(S).')
170   RETURN
      END
C
C
C**********************************************************************
C
C  ROUTINE NAME  -EXPAND
C
C  PURPOSE        -EXPANDS THE FINAL SIMPLEX AROUND THE CENTROID
C                  IN ORDER TO DECREASE THE POSSIBILITY THAT THE
C                  BETAM MATRIX TO BE CALCULATED CONSISTS LARGELY
C                  OF ROUNDOFF ERRORS INCURRED IN CALCULATING THE
C                  RESPONSES.
C
C  REMARKS        -WHEN ONLY 1 PARAMETER NEEDS TO BE OPTIMIZED
C                  A SPECIAL PROCEDURE IS USED IN THE EXPANSION
C                  PROCESS. SINCE IN THAT CASE THE CENTROID
C                  CORRESPONDS TO THE BEST POINT, IT IS THEREFORE
C                  IMPOSSIBLE TO EXPAND THIS POINT AROUND ITSELF.
C                  AN " AVERAGE " POINT IS THUS CALCULATED BETWEEN
C                  THE 2 VERTICES AND TREATED AS THE CENTROID.
C
C  USAGE          -CALL EXPAND(RCSERR)
C
C  ARGUMENTS AND VARIABLES
C
C                       RCSERR -RELATIVE CHI-SQUARE ERROR VALUE
C                                (SEE SUBROUTINE ACCU).
C                          MAX -WORST RESPONSE IN THE OPTIMUM SIMPLEX.
C                        WORST -INDEX OF THE WORST VERTEX IN RESPV.
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```
C                      CENRES -RESPONSE AT THE CENTROID.
C                      EXPLIM -LIMIT OF ENLARGEMENT OF OPTIMUM
C                              SIMPLEX. CALCULATED IN SUBR. ACCU.
C                        TEST -RELATIVE DIFFERENCE BETWEEN ANY VERTEX
C                              RESPONSE AND THE CENTROID RESPONSE.
C                       HOLDV -BUFFER VECTOR USED TO SAVE THE PARAM.
C                              VALUES IN CASE OF FAILURE OF CHECK
C                              TEST.
C                        HOLD -BUFFER SCALAR USED TO SAVE THE
C                              RESPONSE VALUE.
C                        DABS -FORTRAN DOUBLE-PRECISION BUILT-IN
C                              FUNCTION.
C
C
      SUBROUTINE EXPAND(RCSERR)
C
C
C  DIMENSION AM(T2,T3),RESPV(T3),HOLDV(T2).
C
      DIMENSION AM(14,103),RESPV(103),HOLDV(14)
C
      DOUBLE PRECISION AM,RESPV,HOLDV,MAX,CENRES,TEST,HOLD,EXPLIM,
     &                 DABS,RCSERR
C
      INTEGER VMAX,VMAXP1,WORST,CHECKF,ADF,SOLNUM,CONCL,TEMPL,
     &        SOLNP1,IMPNUM
C
      COMMON /COPT1/ AM,RESPV
      COMMON /COPT3/ VMAX,VMAXP1,SOLNUM,SOLNP1,IMPNUM,CONCL,TEMPL
      COMMON /COPT5/ CHECKF,ADF
C
C
C  FIND THE WORST VERTEX IN THE OPTIMUM SIMPLEX.
C
      MAX=0.0D0
      DO 10 K=1,VMAXP1
      IF (.NOT.(RESPV(K) .GT. MAX)) GO TO 10
      MAX=RESPV(K)
      WORST=K
10    CONTINUE
C
C  CALCULATE CENTROID OF ALL MEASUREMENTS EXCEPT WORST ONLY IF
C  OPTIMUM SIMPLEX CONTAINS MORE THAN 2 VERTICES. OTHERWISE USE
C  SPECIAL PROCEDURE TO GET THE CENTROID. ZERO OUT THE CENTROID
C  ELEMENTS FIRST. STORE THESE ELEMENTS IN AM MATRIX.
C
      DO 20 K=1,VMAX
20    AM(K,VMAX+2)=0.0D0
C
C  CHECK IF SPECIAL PROCEDURE IS NEEDED, I.E. VMAX = 1.
C
      IF (VMAX .EQ. 1) GO TO 130
      DO 30 I=1,VMAX
      DO 30 J=1,VMAXP1
30    IF (J .NE. WORST) AM(I,VMAX+2)=AM(I,VMAX+2)+AM(I,J)
C
C  DIVIDE CENTROID DOWN BY VMAX.
C
      DO 40 K=1,VMAX
40    AM(K,VMAX+2)=AM(K,VMAX+2)/VMAX
      GO TO 140
130   CONTINUE
C
C  IN THE CASE WHERE OPTIMUM SIMPLEX CONTAINS ONLY 2 VERTICES,
C  I.E. VMAX = 1, THE CENTROID IS DEFINED SIMPLY AS THE MIDPOINT
C  BETWEEN THESE VERTICES.
C
      DO 150 J=1,VMAXP1
      AM(VMAX,VMAX+2)=AM(VMAX,VMAX+2)+AM(VMAX,J)
150   CONTINUE
      AM(VMAX,VMAX+2)=AM(VMAX,VMAX+2)/VMAXP1
140   CONTINUE
C
C  EVALUATE THE RELATIVE ERROR ATTACHED TO THE EVALUATION OF THE
C  CENTROID RESPONSE VALUE. DETERMINE THE LIMIT OF THE EXPANSION
C  TO BE CARRIED OUT.
C
      CALL ACCU(RCSERR,EXPLIM)
C
C  IF CHECKF HAS BEEN SET TO 1, THE CALCULATED CENTROID IS NOT
C  VALID. GO BACK TO CALLING ROUTINE. IF ADF HAS BEEN SET TO 1,
C  THERE IS A MALFUNCTION OF SUBR. ODE (IN SUBR. CELLCH), GO BACK
C  TO CALLING ROUTINE.
C
      IF ((CHECKF .EQ. 1) .OR. (ADF .EQ. 1)) GO TO 120
C
C  OTHERWISE START THE EXPANSION.
C
      CENRES=RESPV(VMAX+2)
C
C  EXPAND THE FINAL SIMPLEX AROUND THE CENTROID UNTIL THE RESPONSE
C  AT EACH VERTEX EXCEEDS THAT AT THE CENTROID BY MORE THAN EXPLIM.
C  THE EXPANSION IS CARRIED OUT UNTIL EXPLIM IS ACHIEVED OR THE
C  VALUES OF THE PARAMETERS FALL OUT OF THEIR DOMAIN OF VALIDITY.
C  AT THAT POINT THE EXPANSION IS STOPPED AND THE PREVIOUS VERTEX
C  IS USED AS FINAL VALUE.
C
      J=1
100   IF (J .GT. VMAXP1) GO TO 120
      TEST=DABS((RESPV(J)-CENRES)/CENRES)
80    IF (TEST .GT. EXPLIM) GO TO 110
      WRITE(2,2000) J
2000  FORMAT(//,' EXPANSION OF SIMPLEX IN PROGRESS. VERTEX: ',I2)
C
C  SAVE RESPV(J) AND CORRESPONDING AM(I,J) VALUES IN CASE OF FAILURE
C  OF CHECK TEST.
C
      HOLD=RESPV(J)
      DO 50 I=1,VMAX
50    HOLDV(I)=AM(I,J)
C
C  DOUBLE DISTANCE BETWEEN CENTROID AND VERTEX J.
C
      DO 60 I=1,VMAX
60    AM(I,J)=AM(I,VMAX+2)-2.0D0*(AM(I,VMAX+2)-AM(I,J))
C
C  GET RESPONSE AT NEW VERTEX.
C
      CALL MEAS(J)
C
```

```
C  CHECK IF NEW VERTEX PARAMETER VALUES ARE VALID. IF NOT KEEP
C  PREVIOUS VALUES AND TERMINATE THE EXPANSION IN THAT DIRECTION.
C  IF ADF HAS BEEN SET TO 1, END PROCEDURE AND GO BACK TO CALLING
C  ROUTINE.
C
      IF (ADF .EQ. 1) GO TO 120
      IF (CHECKF .NE. 1) GO TO 70
      RESPV(J)=HOLD
      DO 80 I=1,VMAX
80    AM(I,J)=HOLDV(I)
      CHECKF=0
      J=J+1
      GO TO 100
70    CONTINUE
      TEST=DABS((RESPV(J)-CENRES)/CENRES)
      GO TO 90
110   CONTINUE
      J=J+1
      GO TO 100
120   CONTINUE
      RETURN
      END
C
C
C**********************************************************************
C
C  ROUTINE NAME  -QUADOP
C
C  PURPOSE        -THIS SUBROUTINE IS USED TO ESTIMATE THE
C                  INFORMATION MATRIX IN THE NEIGHBOURHOOD OF THE
C                  MINIMUM AND FROM IT, TO LOCATE THE GLOBAL OPTIMUM.
C
C                  THIS MATRIX PERMITS A DIRECT EVALUATION OF
C                  THE VARIANCE-COVARIANCE MAT. OF THE ESTIMATES.
C                  NOTE THAT THE INFORMATION MATRIX IS EQUIVALENT
C                  TO THE HESSIAN MATRIX, I.E., THE MATRIX OF
C                  SECOND PARTIAL DERIVATIVES OF THE QUADRATIC
C                  MODEL, FROM WHICH WE CAN STUDY THE NATURE OF
C                  THE STATIONARY POINT.
C
C  USAGE          -CALL QUADOP(IERROR)
C
C  ARGUMENTS AND VARIABLES
C
C              IERROR -ERROR FLAG TO SIGNAL MALFUNCTION OF UTILITY
C                      ROUTINE GAUSS.
C              MPTCNT -INDEX OF THE POSITION IN AM MATRIX (COLUMN #)
C                      OF THE MIDPOINT VERTICES GENERATED FOR THE
C                      QUADRATIC APPROXIMATION.
C               RESPM -SYMMETRICAL MATRIX OF THE CHI-SQUARE VALUES
C                      CORRESPONDING TO EACH VERTEX OF AM MATRIX.
C                  OM -THE TRANSFORMATION MATRIX TO GO FROM OLD
C                      SYSTEM OF COORDINATES TO NEW ONE AND VICE-VERSA
C       ALPHAV,BETAM -THE FIRST-ORDER PARTIAL DERIVATIVES VECTOR
C                      AND INFORMATION MATRIX (HESSIAN) RESPECTIVELY.
C              BETAIM -INVERSE OF BETAM
C                  ZV -VECTOR OF THE STATIONARY POINT PARAMETER VALUES
C         HOLD,HOLDV -BUFFER SCALAR AND VECTOR USED TO SAVE RESPV
C                      AND AM ELEMENTS IN THE SORTING PROCEDURE.
C        BUFFV,BUFFM -BUFFER ARRAYS USED IN GAUSS AS INPUT/OUTPUT.
C
C  PROGRAMMING NOTES
C
C                 SUBROUTINE GAUSS IS USED TO SOLVE THE SYSTEM
C                 [ BETAM ][ -ZV ]=[ ALPHAV ] WHERE [ ZV ]
C                 IS THE VECTOR OF THE TRUE OPTIMUM PARAMETERS. THE
C                 INVERSE OF BETAM IS ALSO CALCULATED. SEE THE
C                 ROUTINE FOR DETAILS ON THE ALGORITHM AND ON ITS
C                 USAGE.
C
C
C
      SUBROUTINE QUADOP(IERROR)
C
C
C  DIMENSION AM(T2,T3),RESPV(T3),RESPM(T2,T2),OM(T2,T2),
C            ALPHAV(T2),BETAM(T2,T2),ZV(T2),HOLDV(T2),BUFFV(T2),
C            BUFFM(T2,T2),WORK((T2+1)*T2),IWORK(T2),BETAIM(T2,T2)
C
      DIMENSION AM(14,103),RESPV(103),RESPM(14,14),OM(14,14),
     @          ALPHAV(14),BETAM(14,14),ZV(14),HOLDV(14),BUFFV(14),
     $          BUFFM(14,14),WORK(210),IWORK(14),BETAIM(14,14)
C
      DOUBLE PRECISION AM,RESPV,RESPM,OM,ALPHAV,BETAM,ZV,
     &                 HOLD,HOLDV,BUFFV,BUFFM,DT,WORK,BETAIM
C
      INTEGER MPTCNT,VMAX,VMAXP1,WORST,NEWPNT,TOTCNT,
     @        CHECKF,ADF,IJOB,N,NDIM,IER,KP1,JP1,IP1,SOLNUM,SOLNP1,
     @        IMPNUM,IWORK,BEST,IERROR,CONCL,TEMPL
C
      COMMON /COPT1/ AM,RESPV
      COMMON /COPT3/ VMAX,VMAXP1,SOLNUM,SOLNP1,IMPNUM,CONCL,TEMPL
      COMMON /COPT4/ BEST,WORST,NEWPNT,TOTCNT
      COMMON /COPT6/ CHECKF,ADF
      COMMON /COPT7/ OM,ALPHAV,BETAM,BETAIM
C
C
      WRITE(2,2000)
2000  FORMAT(//,' >>>>>>>  QUADRATIC APPROXIMATION IN PROGRESS.')
C
C  FROM FINAL SIMPLEX, GET OM TAKING THE VERTEX WITH THE BEST
C  RESPONSE AS ORIGIN.
C
C  REARRANGE FINAL AM MATRIX IN ORDER TO HAVE THE VERTICES IN
C  INCREASING ORDER OF RESPONSE VALUES (BEST TO WORST). THE FIRST
C  COLUMN WILL THEN BE DEFINED AS THE ORIGIN VERTEX.
C
C  SORT RESPV IN INCREASING ORDER OF CHI-SQUARE VALUES, MOVE THE
C  AM MATRIX COLUMNS ACCORDINGLY.
C
      DO 10 K=1,VMAX
      KP1=K+1
      DO 10 L=KP1,VMAXP1
      IF (RESPV(K) .LE. RESPV(L)) GO TO 10
      HOLD=RESPV(L)
      DO 20 I=1,VMAX
20    HOLDV(I)=AM(I,L)
C
C  MOVE THE VARIABLES OF COLUMN K OF AM IN COLUMN L, MOVE RESPONSE
C  K IN L POSITION IN RESPV.
C
```

```
      CALL MOVE(L,K)
      RESPV(K)=HOLD
      DO 30 I=1,VMAX
30    AM(I,K)=HOLDV(I)
10    CONTINUE
C
C  GENERATE THE TRANSFORMATION MATRIX OM. THE FIRST COLUMN OF AM
C  IS THE REFERENCE VERTEX.
C
      DO 40 I=1,VMAX
      DO 40 J=1,VMAX
40    OM(I,J)=AM(I,J+1)-AM(I,1)
C
C  CALCULATE THE COORDINATES OF THE 0.5*N*(N+1) MIDPOINTS NEEDED
C  TO GENERATE THE QUADRATIC SURFACE. CALCULATE THE RESPONSE FOR
C  THESE HALFPOINTS.
C
      MPTCNT=VMAX+2
      DO 50 J=1,VMAX
      JP1=J+1
      DO 50 K=JP1,VMAXP1
      DO 60 I=1,VMAX
60    AM(I,MPTCNT)=(AM(I,J)+AM(I,K))/2.0D0
      CALL MEAS(MPTCNT)
C
C  IF CHECKF AND/OR ADF HAVE BEEN SET TO 1, END PROCEDURE AND GO
C  BACK TO CALLING ROUTINE.
C
      IF ((CHECKF .EQ. 1) .OR. (ADF .EQ. 1)) GO TO 180
      MPTCNT=MPTCNT+1
50    CONTINUE
C
C  RESHAPE RESPV IN A SYMMETRICAL RESPONSE MATRIX RESPM.
C
      DO 70  K=1,VMAXP1
70    RESPM(K,K)=RESPV(K)
      MPTCNT=VMAX+2
      DO 80 I=1,VMAX
      IP1=I+1
      DO 80 J=IP1,VMAXP1
      RESPM(I,J)=RESPV(MPTCNT)
      RESPM(J,I)=RESPM(I,J)
      MPTCNT=MPTCNT+1
      TOTCNT=MPTCNT-1
80    CONTINUE
C
C  CALCULATE ALPHAV AND BETAM COEFFICIENTS. ASSEMBLE GRADIENT
C  VECTOR AND HESSIAN MATRIX.
C
      DO 90 K=1,VMAX
      ALPHAV(K)=(4.0D0*RESPM(1,K+1))-RESPM(K+1,K+1)-
     &                                    (3.0D0*RESPM(1,1))
90    BETAM(K,K)=4.0D0*(RESPM(K+1,K+1)+RESPM(1,1)-
     &                                    (2.0D0*RESPM(1,K+1)))
      DO 100 I=1,VMAX
      IP1=I+1
      DO 100 J=IP1,VMAXP1
      BETAM(I,J)=4.0D0*(RESPM(I+1,J+1)+RESPM(1,1)-RESPM(1,I+1)
     &                                        -RESPM(1,J+1))
100   BETAM(J,I)=BETAM(I,J)
C
C  EVALUATE THE TRUE OPTIMUM. GET ZV PARAMETERS AND INVERSE OF
C  BETAM. SET PARAMETERS NEEDED BY ROUTINE GAUSS.
C
C  GENERATE INTERMEDIATE ARRAYS TO BE USED AS INPUT/OUTPUT IN
C  SUBR. GAUSS.
C
      DO 110 K=1,VMAX
110   BUFFV(K)=ALPHAV(K)
      DO 120 I=1,VMAX
      DO 120 J=1,VMAX
120   BUFFM(I,J)=BETAM(I,J)
      IERROR=0
      IJOB=3
      N=VMAX
      NDIM=14
      CALL GAUSS(NDIM,N,IJOB,BUFFM,BUFFV,DT,IWORK,WORK,IER)
C
C  PRINT ERROR MESSAGE IF MATRIX IS SINGULAR OR IF IJOB VAR. NOT
C  WELL DEFINED AND STOP THE WHOLE PROGRAM.
C
      IF ((IER .NE. 1) .AND. (IER .NE. 2)) GO TO 130
      IF (IER .EQ. 1) GO TO 125
      WRITE(2,2010)
2010  FORMAT(//,' ***** MATRIX IS SINGULAR (BETA) *****')
      IERROR=1
      RETURN
125   CONTINUE
      WRITE(2,2020)
2020  FORMAT(//,' ***** IJOB NOT WELL DEFINED (QUADOP) *****')
      IERROR=1
      RETURN
130   CONTINUE
C
C  OTHERWISE GET THE TRUE OPTIMUM. GET BETAIM.
C
      DO 140 K=1,VMAX
140   ZV(K)=-BUFFV(K)
      DO 150 I=1,VMAX
      DO 150 J=1,VMAX
150   BETAIM(I,J)=BUFFM(I,J)
C
C  CONVERT ZV VAL. BACK TO OLD COORDINATES SYSTEM.
C
      DO 160 I=1,VMAX
      AM(I,TOTCNT+1)=0.0D0
      DO 170 J=1,VMAX
170   AM(I,TOTCNT+1)=AM(I,TOTCNT+1)+OM(I,J)*ZV(J)
160   AM(I,TOTCNT+1)=AM(I,1)+AM(I,TOTCNT+1)
180   RETURN
      END
C
C
C*****************************************************************
C
C  ROUTINE NAME  -DETERM
C
C  PURPOSE        -ANALYSES USING THE INFORMATION MATRIX BETAM, WHICH
C                  IS EQUIVALENT TO THE HESSIAN MATRIX, THE CHARACTER
C                  OF THE STATIONARY POINT CALCULATED IN SUBR QUADOP.
```

```
C
C                   IN ORDER TO VERIFY IF THE OPTIMUM IS A TRUE MINIM.
C                   THE DETERMINANTS OF EACH OF THE PRINCIPAL MINORS
C                   OF THE HESSIAN MATRIX ARE CALCULATED. IF EACH OF
C                   THESE MINORS HAS A DETERMINANT .GT. 0, THEN THE
C                   RESPONSE AT THE STATIONARY POINT IS A MINIMUM.
C
C                   OTHERWISE DETRF IS SET TO 1 AND THE SIMPLEX
C                   SEARCH CONTINUES WITH MORE STRICT TOLSPX AND
C                   TOLAVE VALUES USING THE PREVIOUSLY OBTAINED OPTIM.
C                   VERTEX AS STARTING GUESS OF THE PARAMETERS. THIS
C                   PROCESS IS CARRIED OUT UNTIL A TRUE MINIMUM IS
C                   OBTAINED OR UNTIL SPXMAX CYCLES ARE PERFORMED.
C
C  USAGE           -CALL DETERM(DETRF,IERROR)
C
C  ARGUMENTS AND VARIABLES
C
C                   DETRF -FLAG USED TO INDICATE IF THE BETAM MATRIX
C                          IS POSITIVE-DEFINITE (DETRF=1 IF NOT).
C                  IERROR -ERROR FLAG TO SIGNAL MALFUNCTION OF UTILITY
C                          ROUTINE GAUSS.
C                  MINORV -VECTOR OF THE DETERMINANTS OF THE PRINCIPAL
C                          MINORS OF BETAM.
C                   BUFFM -BUFFER MAT. USED IN GAUSS AS INPUT/OUTPUT.
C
C  PROGRAMMING NOTES
C
C                SUBROUTINE GAUSS IS USED TO CALCULATE THE
C                DETERMINANTS OF THE PRINCIPAL MINORS OF BETAM.
C                SEE THE ROUTINE FOR DETAILS ON THE ALGORITHM AND
C                ON ITS USAGE.
C
C
      SUBROUTINE DETERM(DETRF,IERROR)
C
C
C  DIMENSION BETAM(T2,T2),MINORV(T2),BUFFM(T2,T2),OM(T2,T2),
C            ALPHAV(T2),B(T2),WORK(T2),IWORK(T2),BETAIM(T2,T2).
C
      DIMENSION BETAM(14,14),MINORV(14),BUFFM(14,14),OM(14,14),
     @          ALPHAV(14),B(14),WORK(14),IWORK(14),BETAIM(14,14)
C
      DOUBLE PRECISION BETAM,MINORV,BUFFM,OM,ALPHAV,B,WORK,DT,
     @                 BETAIM
C
      INTEGER DETRF,VMAX,VMAXP1,IJOB,N,NDIM,IER,SOLNUM,SOLNP1,
     @        IMPNUM,IWORK,IERROR,CONCL,TEMPL
C
      COMMON /COPT3/ VMAX,VMAXP1,SOLNUM,SOLNP1,IMPNUM,CONCL,TEMPL
      COMMON /COPT7/ OM,ALPHAV,BETAM,BETAIM
C
C
      WRITE(2,2000)
2000  FORMAT(//,' >>>>>>  MINORS OF THE HESSIAN MATRIX',
     @       ' ARE BEING CHECKED.')
C
C  USING SUBR. GAUSS CALCULATE THE DETERMINANTS OF THE PRINCIPAL
C  MINORS OF THE HESSIAN MATRIX. BUFFM CONTAINS AS INPUT A SQUARE
C  MATRIX OF ORDER K. THE VALUE OF THE DETERMINANT OF THAT MATRIX
C  IS PUT AS AN ELEMENT OF MINORV.
C
C  SET PARAMETERS NEEDED ROUTINE BY ROUTINE GAUSS. SET DETRF TO 0.
C
      DETRF=0
      K=VMAX
50    IF (K .LT. 1) GO TO 80
C
C  SET UP BUFFM.
C
      I=K
30    IF (I .LT. 1) GO TO 40
      J=K
10    IF (J .LT. 1) GO TO 20
      BUFFM(I,J)=BETAM(I,J)
      J=J-1
      GO TO 10
20    CONTINUE
      I=I-1
      GO TO 30
40    CONTINUE
      IERROR=0
      IJOB=4
      N=K
      NDIM=14
      CALL GAUSS(NDIM,N,IJOB,BUFFM,B,DT,IWORK,WORK,IER)
C
C  PRINT ERROR MESSAGE IF MATRIX IS SINGULAR OR IF IJOB NOT WELL
C  DEFINED AND STOP THE WHOLE PROGRAM.
C
      IF ((IER .NE. 1) .AND. (IER .NE. 2)) GO TO 60
      IF (IER .EQ. 1) GO TO 75
      WRITE(2,2010)
2010  FORMAT(//,' ***** MATRIX IS SINGULAR (MINOR OF BETAM) *****')
      IERROR=1
      RETURN
75    CONTINUE
      WRITE(2,2020)
2020  FORMAT(//,' ***** IJOB NOT WELL DEFINED (DETERM) *****')
      IERROR=1
      RETURN
60    CONTINUE
C
C  GET THE VALUE OF THE DETERMINANT.
C
      MINORV(K)=DT
      K=K-1
      GO TO 50
80    CONTINUE
C
C  CHECK IF ANY DETERMINANT IS NEGATIVE.
C
      DO 70 K=1,VMAX
      IF (MINORV(K) .GT. 0.0D0) GO TO 70
      WRITE(2,2030) K,MINORV(K)
2030  FORMAT(/,' ***** DETERMINANT IS NEGATIVE. NOT A TRUE MINIMUM',
     @       ' *****  DETERMINANT: ',I2,' ',1PD10.3)
      DETRF=1
70    CONTINUE
      RETURN
      END
C
C
C***********************************************************
C
C ROUTINE NAME   -STERR
C
C  PURPOSE        -USES THE INFORMATION MATRIX BETAM GENERATED IN
C                  QUADOP TO ESTIMATE THE STANDARD ERRORS OF THE
C                  OPTIMIZED PARAMETERS.
C
C  USAGE          -CALL STERR(STERRV,IERROR)
C
C  ARGUMENTS AND VARIABLES
C
C                 STERRV -VECTOR OF THE STANDARD ERRORS OF THE FINAL
C                         OPTIMIZED PARAMETERS.
C                 IERROR -ERROR FLAG TO SIGNAL MALFUNCTION OF
C                         UTILITY ROUTINES GAUSS, MULAB AND MULABT.
C                 ABETAM -MATRIX OF THE ABSOLUTE VALUE OF BETAM.
C                 ABETIM -INVERSE OF ABETAM.
C                 INTERM -INTERMEDIATE MATRIX USED IN THE CALC. OF
C                         THE INFORMATION MATRIX, INFOM.
C                  INFOM -INFORMATION MATRIX (HESSIAN MATRIX) IN
C                         TERMS OF ORIGINAL COORDINATE SYSTEM.
C                 VARCOM -VARIANCE-COVARIANCE MATRIX.
C                 INTERV -INTERMEDIATE VECTOR USED IN CALC. OF THE
C                         RESPONSE AT OPTIMUM.
C                  BUFFM -BUFFER MATRIX USED IN THE CALC. OF ABETIM.
C             DABS,DSQRT -FORTRAN DOUBLE-PRECISION BUILT-IN FUNC.
C
C  PROGRAMMING NOTES
C
C                 SUBROUTINE GAUSS IS USED TO CALCULATE THE INVERSE
C                 OF ABETAM. SUBR. MULAB AND MULABT ARE USED TO
C                 PERFORM MATRIX MULTIPLICATIONS. SEE THE ROUTINES
C                 FOR DETAILS ON THE ALGORITHMS AND ON THEIR USAGE.
C
C

      SUBROUTINE STERR(STERRV,IERROR)

C
C
C  DIMENSION AM(T2,T3),RESPV(T3),OM(T2,T2),ALPHAV(T2),
C            BETAM(T2,T2),BETAIM(T2,T2),STERRV(T2),ABETAM(T2,T2),
C            ABETIM(T2,T2),INTERM(T2,T2),INFOM(T2,T2),VARCOM(T2,T2),
C            INTERV(T2),BUFFM(T2,T2),B(T2),WORK((T2+1)*T2),
C            IWORK(T2),YV(T4),WTV(T4),XV(T4),CASEM(T6,T6).
C
      DIMENSION AM(14,103),RESPV(103),OM(14,14),ALPHAV(14),
     @          BETAM(14,14),BETAIM(14,14),STERRV(14),ABETAM(14,14),
     @          ABETIM(14,14),INTERM(14,14),VARCOM(14,14),
     @          INTERV(14),BUFFM(14,14),B(14),WORK(210),IWORK(14),
     @          YV(4000),WTV(4000),XV(4000),CASEM(20,8),INFOM(14,14)
C
      DOUBLE PRECISION AM,RESPV,OM,ALPHAV,BETAM,BETAIM,STERRV,
     @                 ABETAM,ABETIM,INTERM,VARCOM,INTERV,BUFFM,
     @                 B,WORK,DT,DABS,DSQRT,YV,WTV,XV,INFOM
C
      INTEGER VMAX,VMAXP1,WORST,NEWPNT,TOTCNT,IJOB,N,NDIM,IER,
     @        L,M,IB,IC,SOLNUM,SOLNP1,IMPNUM,IA,IWORK,BEST,IERROR,
     @        CONCL,TEMPL,CASEM,TOTCAS
C
C  EXECUTION-TIME FORMAT STATEMENTS.
C  DIMENSION DIGIT(T2)/'1',...,'T2'/
C
      INTEGER DIGIT(14)/'1','2','3','4','5','6','7','8','9','10',
     @                  '11','12','13','14'/
      INTEGER VF(4)/'(1P','    ','D14.','4)'/
C
      COMMON /COPT1/ AM,RESPV
      COMMON /COPT2/ YV,WTV,XV,CASEM
      COMMON /COPT3/ VMAX,VMAXP1,SOLNUM,SOLNP1,IMPNUM,CONCL,TEMPL
      COMMON /COPT4/ BEST,WORST,NEWPNT,TOTCNT
      COMMON /COPT7/ OM,ALPHAV,BETAM,BETAIM
C
C
C  GET THE INVERSE OF THE ABSOLUTE VALUE OF BETAM, ABETIM.
C
      DO 10 I=1,VMAX
      DO 10 J=1,VMAX
10    ABETAM(I,J)=DABS(BETAM(I,J))
C
C  SET UP PARAMETERS NEEDED BY SUBR. GAUSS.
C
      DO 20 I=1,VMAX
      DO 20 J=1,VMAX
20    BUFFM(I,J)=ABETAM(I,J)
      IERROR=0
      IJOB=1
      N=VMAX
      NDIM=14
      CALL GAUSS(NDIM,N,IJOB,BUFFM,B,DT,IWORK,WORK,IER)
C
C  PRINT ERROR MESSAGE IF MATRIX IS SINGULAR OR IF IJOB NOT WELL
C  DEFINED AND STOP THE WHOLE PROGRAM.
C
      IF ((IER .NE. 1) .AND. (IER .NE. 2)) GO TO 30
      IF (IER .EQ. 1) GO TO 25
      WRITE(2,2000)
2000  FORMAT(//,' ***** MATRIX IS SINGULAR (ABETAM) *****')
      IERROR=1
      RETURN
25    CONTINUE
      WRITE(2,2010)
2010  FORMAT(//,' ***** IJOB NOT WELL DEFINED (STERR) *****')
      IERROR=1
      RETURN
30    CONTINUE
C
C  SET ABETIM. THEN MULTIPLY FIRST OM BY ABETIM, GENERATE INTERM.
C  THEN MULTIPLY INTERM BY THE TRANSPOSE OF OM, GENERATE INFOM.
C
      DO 40 I=1,VMAX
      DO 40 J=1,VMAX
40    ABETIM(I,J)=BUFFM(I,J)
C
C  SET UP PARAMETERS NEEDED BY SUBR. MULAB AND MULABT.
C
      L=VMAX
      M=VMAX
      N=VMAX
      IA=14
      IB=14
      IC=14
      CALL MULAB(OM,ABETIM,L,M,N,IA,IB,INTERM,IC,IER)
      IF (IER .NE. 1) GO TO 45
      WRITE(2,2020)
2020  FORMAT(//,' **** MATRIX DIMENSIONED INCORRECTLY (STERR) ****')
      IERROR=1
      RETURN
45    CONTINUE
      CALL MULABT(INTERM,OM,L,M,N,IA,IB,INFOM,IC,IER)
      IF (IER .NE. 1) GO TO 55
      WRITE(2,2030)
2030  FORMAT(//,' **** MATRIX DIMENSIONED INCORRECTLY (STERR) ****')
      IERROR=1
      RETURN
55    CONTINUE
C
C  GET THE RESPONSE AT THE OPTIMUM. MULTIPLY FIRST THE TRANSPOSE
C  OF ALPHAV BY BETAIM, GENERATE INTERV.
C
      DO 50 J=1,VMAX
      INTERV(J)=0.0D0
      DO 50 I=1,VMAX
50    INTERV(J)=INTERV(J)+(ALPHAV(I)*BETAIM(I,J))
C
C  THEN MULTIPLY INTERV BY ALPHAV. GET THE RESPONSE AT OPTIMUM.
C
      RESPV(TOTCNT+1)=0.0D0
      DO 60 K=1,VMAX
60    RESPV(TOTCNT+1)=RESPV(TOTCNT+1)+(INTERV(K)*ALPHAV(K))
      RESPV(TOTCNT+1)=RESPV(1)-(0.5D0*RESPV(TOTCNT+1))
C
C  GET TOTAL NUMBER OF EXPERIMENTAL POINTS.
C
      TOTCAS=0
      DO 80 J=1,TEMPL
      DO 80 I=1,CONCL
      TOTCAS=TOTCAS+CASEM(I,J)
80    CONTINUE
C
C  GENERATE VARIANCE-COVARIANCE MATRIX, VARCOM.
C
      DO 85 J=1,VMAX
      DO 85 I=1,VMAX
      VARCOM(I,J)=2.0D0*(RESPV(TOTCNT+1)/(TOTCAS-VMAX))*
     @            INFOM(I,J)
85    CONTINUE
C
C  GET THE STANDARD ERRORS OF THE ESTIMATED PARAMETERS, STERRV.
C
      DO 70 K=1,VMAX
70    STERRV(K)=DSQRT(VARCOM(K,K))
      WRITE(2,2040)
2040  FORMAT(//,' VARIANCE-COVARIANCE MATRIX OF ESTIMATES.',/)
      VF(2)=DIGIT(VMAX)
      WRITE(2,VF) ((VARCOM(I,J),J=1,VMAX),I=1,VMAX)
      RETURN
      END
C
C
C*********************************************************************
C
C  ROUTINE NAME   -ACCU
C
C  PURPOSE        -THIS SUBROUTINE ESTIMATES THE GLOBAL TRUNCATION
C                  ERRORS INCURRED IN COMPUTING THE PREDICTED
C                  RELATIVE CELL VOLUME VALUES (SEE SUBR. CELLCH).
C                  IT DETERMINES THE EXTENT OF THE EXPANSION
C                  TO BE PERFORMED IN SUBR. EXPAND TO ENSURE THAT
C                  ACCEPTABLE ESTIMATES OF THE BETAM ELEMENTS WILL
C                  BE OBTAINED.
C
C  USAGE          -CALL ACCU(RCSERR,EXPLIM)
C
C  ARGUMENTS AND VARIABLES
C
C                 RCSERR -RELATIVE CHI-SQUARE ERROR VALUE, USED IN
C                         ESTIMATING SPXMAX. THIS IS A MEASURE OF
C                         THE RELATIVE ACCURACY TO BE EXPECTED, ON
C                         AVERAGE, ON ANY CHI-SQUARE VALUE CALC.
C                         IN THE PROGRAM.
C                 EXPLIM -LIMIT OF EXPANSION OF OPTIMUM SIMPLEX TO
C                         BE PERFORMED IN SUBR. EXPAND.
C                   SUM1 -SUM OF THE ABSOLUTE VALUES OF THE WEIGHTED
C                         RESIDUALS. USED IN ESTIMATING RCSERR.
C                   SUM2 -SUM OF THE SQUARES OF THE RESIDUALS. USED
C                         TO CALCULATE THE CENTROID RESPONSE.
C          CALC1V,CALC2V -VECTORS OF THE PREDICTED RELATIVE CELL
C                         VOLUME VALUES.
C                 CSQERR -MAXIMUM ERROR INCURRED IN EVALUATING THE
C                         CHI-SQUARE VALUES USED IN THE OPTIMIZATION
C                 ERRORV -VECTOR OF THE ESTIMATED GLOBAL TRUNCATION
C                         ERRORS.
C                   DABS -FORTRAN DOUBLE-PRECISION BUILT-IN FUNC.
C
C
      SUBROUTINE ACCU(RCSERR,EXPLIM)
C
C
C  DIMENSION AM(T2,T3),RESPV(T3),YV(T4),WTV(T4),XV(T4),CASEM(T5,T6),
C           ERRORV(T7),CALC1V(T7),CALC2V(T7).
C
      DIMENSION AM(14,103),RESPV(103),YV(4000),WTV(4000),XV(4000),
     @          CASEM(20,8),ERRORV(120),CALC1V(120),CALC2V(120)
C
      DOUBLE PRECISION AM,RESPV,YV,WTV,XV,RCSERR,EXPLIM,ERRORV,
     @                 CALC1V,CALC2V,TOLABS,TOLREL,SUM1,CSQERR,DABS,
     @                 TOLA,TOLR,SUM2,INTERM
C
      INTEGER VMAX,VMAXP1,CHECKF,ADF,SOLNUM,SOLNP1,CONCL,TEMPL,
     @        IMPNUM,CASEM,NCOUNT,TIND,CLIND,AMCIND,NCASES,NCTIND
C
      COMMON /COPT1/ AM,RESPV
      COMMON /COPT2/ YV,WTV,XV,CASEM
      COMMON /COPT3/ VMAX,VMAXP1,SOLNUM,SOLNP1,IMPNUM,CONCL,TEMPL
      COMMON /COPT5/ CHECKF,ADF
      COMMON /CCELL4/ TOLABS,TOLREL
      COMMON /CCELL5/ TIND,CLIND,AMCIND,NCTIND
```

```
C
C
      NCOUNT=1
      SUM1=0.0D0
      SUM2=0.0D0
      DO 10 J=1,TEMPL
      DO 10 I=1,CONCL
      IF (CASEM(I,J) .EQ. 0) GO TO 10
      TIND=J
      CLIND=I
      AMCIND=VMAX+2
      NCTIND=NCOUNT
C
C  PERFORM THE INTEGRATION. COMPUTE VECTOR OF PREDICTED RELATIVE
C  CELL VOLUME VALUES (CALC1V) USING THE MOST RECENT CENTROID VERTEX
C  PARAMETERS FOR THE GIVEN TEMPERATURE-CONCENTRATION COMBINATION.
C
      CALL CELLCH(CALC1V)
C
C  IF CHECKF AND/OR ADF HAVE BEEN SET TO 1, END PROCEDURE AND GO
C  BACK TO CALLING ROUTINE.
C
      IF ((CHECKF .EQ. 1) .OR. (ADF .EQ. 1)) RETURN
C
C  GET AN ESTIMATE OF THE GLOBAL TRUNCATION ERROR MADE ON EACH OF
C  THE PREDICTED RELATIVE CELL VOLUME VALUES. PERFORM THE
C  INTEGRATION WITH REDUCED TOLERANCES (SET TOLABS=0.1*TOLABS  AND
C  TOLREL=0.1*TOLREL).
C
C  SAVE ORIGINAL TOLERANCES VALUES.
C
      TOLA=TOLABS
      TOLR=TOLREL
      TOLABS=0.1D0*TOLABS
      TOLREL=0.1D0*TOLREL
      CALL CELLCH(CALC2V)
      IF ((CHECKF .EQ. 1) .OR. (ADF .EQ. 1)) RETURN
C
C  RESTORE TOLERANCE VALUES.
C
      TOLABS=TOLA
      TOLREL=TOLR
C
      NCASES=CASEM(I,J)
      DO 10 K=1,NCASES
      ERRORV(K)=CALC1V(K)-CALC2V(K)
C
C  GET RESPONSE OF CURRENT CENTROID SIMULTANEOUSLY.
C
      SUM1=SUM1+DABS(WTV(NCOUNT)*ERRORV(K)*(YV(NCOUNT)-CALC1V(K)))
      SUM2=SUM2+(WTV(NCOUNT)*((YV(NCOUNT)-CALC1V(K))**2))
      NCOUNT=NCOUNT+1
10    CONTINUE
C
C  ESTIMATE THE RELATIVE CHI-SQUARE ERROR.
C
      CSQERR=2.0D0*SUM1
      RESPV(VMAX+2)=SUM2
      RCSERR=CSQERR/RESPV(VMAX+2)
C
C  ESTIMATE THE LIMIT OF EXPANSION OF THE OPTIMUM SIMPLEX (EXPLIM)
C  IN SUBR. EXPAND. THIS LIMIT MUST BE SET TO AT LEAST 1000 TIMES
C  THE MAXIMUM RELATIVE ERROR TO BE EXPECTED IN EVALUATING THE
C  CHI-SQUARE VALUES.
C
      EXPLIM=1000.0D0*RCSERR
      WRITE(2,2000) RCSERR, EXPLIM
2000  FORMAT(///,' MAXIMUM RELATIVE ERROR IN CHI-SQUARE',
     @       ' CALCULATIONS: ',1PD10.3,/,' LIMIT OF EXPANSION',
     @       ' OF OPTIMUM SIMPLEX: ',D10.3)
      RETURN
      END
```

13.2.4 The "utility" routines

This unit contains utility routines which are called either by "cell behavior" subroutines or "optimization" subroutines to perform specific tasks. Subroutines ODE, DE, STEP and INTRP are, for example, part of a *predictor-corrector* integration algorithm (Shampine and Gordon, 1975) called by subroutine CELLCH (see section 13.2.2) to integrate a system of ordinary differential equations. Subroutines GAUSS, INVERT, LNSINV, DET, DECOMP and SOLVE, on their side, are part of a general in place matrix inversion and/or linear equations solver or determinant evaluation algorithm (Forsythe, Malcolm and Moler, 1977; Forsythe and Moler, 1967) which is used in routines QUADOP, DETERM and STERR (see section 13.2.3). Finally, subroutines MULAB, MULABT and MULATB are called by routine STERR to perform matrix multiplications.

```
C***********************************************************************
C
C  THIS FILE CONTAINS UTILITY ROUTINES NEEDED BY OPTIMIZATION
C  PROGRAM MCPERM.
C
C      1.   ROUTINES ODE, DE, STEP AND INTRP ARE PART OF A
C           PREDICTOR-CORRECTOR INTEGRATION ALGORITHM (ADAMS PECE)
C           USED TO GENERATE THE PREDICTED RELATIVE CELL VOLUME
C           VALUES (SEE SUBROUTINE CELLCH).
C
C      2.   ROUTINES GAUSS, INVERT, LNSINV, DET, DECOMP AND SOLVE
C           ARE PART OF A GENERAL IN PLACE MATRIX INVERSION AND/OR
C           LINEAR EQUATIONS SOLVER OR DETERMINANT EVALUATION
C           ALGORITHM.
C
C      3.   ROUTINES MULAB, MULABT AND MULATB ARE THREE MATRIX
C           MULTIPLICATION ROUTINES PERFORMING THE FOLLOWING
C           CALCULATIONS:
C
C                   MULAB   - C = A * B
C                  MULABT   - C = A * (B-TRANSPOSE)
C                  MULATB   - C = (A-TRANSPOSE) * B
C
C***********************************************************************
C
C                        UTILITY ROUTINES 1.
C
C***********************************************************************
C
C  THE FOLLOWING ROUTINES ARE DIRECTLY TAKEN FROM SHAMPINE AND
C  GORDON (SEE ROUTINE DESCRIPTIONS FOR COMPLETE REFERENCE).
C  THEY ARE THE DOUBLE-PRECISION VERSIONS OF THE ORIGINAL CODES
C  AND HAVE BEEN IMPLPMENTED, FOLLOWING THE AUTHORS SUGGESTIONS,
C  TO MAKE THEM MORE ROBUST.
C
C  IN ORDER TO USE THEM FOR THE PROBLEM UNDER STUDY (NUMERICAL
C  ESTIMATION OF MODIFIED PHENOMENOLOGICAL COEFFICIENTS FROM
C  EXPERIMENTAL RELATIVE CELL VOLUME CHANGES, PROGRAM MCPERM), IT
C  WAS NECESSARY TO ADD AN ARGUMENT TO THE PARAMETER LIST OF THE
C  USER PROVIDED SUBROUTINE F, THE ROUTINE STEP AND THE ROUTINE DE.
C
C  SINCE THE MODIFIED PHENOMENOLOGICAL COEFFICIENTS TO BE USED IN
C  THE ROUTINE FLUXES (CALLED FROM F (ADFUN)) ARE TESTED FOR THEIR
C  THEORETICAL VALIDITY WITHIN ADFUN , A TERMINATION FLAG (ISTOP)
C  IS USED IN CASE OF FAILURE OF THE TESTS. IN THAT LATTER CASE
C  ISTOP IS SET TO 1 AND STOPS AUTOMATICALLY THE INTEGRATION
C  PROCESS. CONTROL IS SENT BACK TO THE CALLING ROUTINE (CELLCH).
C
C  IN ORDER TO KEEP THE DESCRIPTIONS OF THE ROUTINES BELOW IN THEIR
C  ORIGINAL FORMS, THIS SPECIAL CHANGE IS NOT DESCRIBED. THE USER
C  SHOULC NEVERTHELESS BE AWARE OF IT IN COMPARING WITH THE
C  REFERENCE CODES.
C
C***********************************************************************
C
C   **********   LATEST VERSION SEPTEMBER 26, 1983   **********
C                      BY CHRISTIAN TOUPIN.
C
C***********************************************************************
C
      SUBROUTINE ODE(F,NEQN,Y,T,TOUT,RELERR,ABSERR,IFLAG,WORK,IWORK)
C
C  SUBROUTINE ODE INTEGRATES A SYSTEM OF NEQN FIRST ORDER
C  ORDINARY DIFFERENTIAL EQUATIONS OF THE FORM
C             DY(I)/DT=F(T,Y(1),Y(2),...,Y(NEQN))
C             Y(I) GIVEN AT T.
C  THE SUBROUTINE INTEGRATES FROM T TO TOUT. ON RETURN THE
C  PARAMETERS IN THE CALL LIST ARE SET FOR CONTINUING THE
C  INTEGRATION. THE USER HAS ONLY TO DEFINE A NEW VALUE TOUT
C  AND CALL ODE AGAIN.
C
C  THE DIFFERENTIAL EQUATIONS ARE ACTUALLY SOLVED BY A SUITE OF CODES
C  DE, STEP, AND INTRP. ODE ALLOCATES VIRTUAL STORAGE IN THE
C  ARRAYS WORK AND IWORK AND CALLS DE. DE IS A SUPERVISOR WHICH
C  DIRECTS THE SOLUTION. IT CALLS ON THE ROUTINES STEP AND INTRP
C  TO ADVANCE THE INTEGRATION AND TO INTERPOLATE AT OUTPUT POINTS.
C
C  ********  IMPORTANT NOTICE  ********
C  THIS SUBROUTINE AS WELL AS DE, STEP AND INTRP ARE THE
C  DCUBLE-PRECISION VERSIONS OF THE ORIGINAL CODES (SEE REF.
C  FOR DETAILS).
C  ************************************
C
C  STEP USES A MODIFIED DIVIDED DIFFERENCE FORM OF THE ADAMS
C  PECE FORMULAS AND LOCAL EXTRAPOLATION. IT ADJUSTS THE ORDER
C  AND STEP SIZE TO CONTROL THE LOCAL ERROR PER UNIT STEP
C  IN A GENERALIZED SENSE. NORMALLY EACH CALL TO STEP
C  ADVANCES THE SOLUTION ONE STEP IN THE DIRECTION OF TOUT.
C  FOR REASONS OF EFFICIENCY DE INTEGRATES BEYOND TOUT INTERNALLY,
C  THOUGH NEVER BEYOND T + 10*(TOUT-T), AND CALLS INTRP TO
C  INTERPOLATE THE SOLUTION AT TOUT. AN OPTION IS PROVIDED TO STOP
C  THE INTEGRATION AT TOUT BUT IT SHOULD BE USED ONLY IF IT IS
C  IMPOSSIBLE TO CONTINUE THE INTEGRATION BEYOND TOUT.
C
C  THIS CODE IS COMPLETELY EXPLAINED AND DOCUMENTED IN THE TEXT,
C  COMPUTER SOLUTION OF ORDINARY DIFFERENTIAL EQUATIONS: THE INITIAL
C  VALUE PROBLEM BY L.F. SHAMPINE AND M.K. GORDON, W.H. FREEMAN AND
C  COMPANY, SAN FRANCISCO, 1975.
C
C  THE PARAMETERS FOR ODE ARE:
C     F -- SUBROUTINE F(T,Y,YP) TO EVALUATE DERIVATIVES YP(I)=DY(I)/DT
C     NEQN -- NUMBER OF EQUATIONS TO BE INTEGRATED
C     Y(*) -- SOLUTION VECTOR AT T
C     T -- INDEPENDENT VARIABLE
C     TOUT -- POINT AT WHICH SOLUTION IS DESIRED
C     RELERR,ABSERR -- RELATIVE AND ABSOLUTE ERROR TOLERANCES FOR LOCAL
C          ERROR TEST. AT EACH STEP THE CODE REQUIRES
C            DABS(LOCAL ERROR) .LE. DABS(Y)*RELERR + ABSERR
C          FOR EACH COMPONENT OF THE LOCAL ERROR AND SOLUTION VECTORS
C     IFLAG -- INDICATES STATUS OF INTEGRATION
C     WORK(*),IWORK(*) -- ARRAYS TO HOLD INFORMATION INTERNAL TO
C          CODE WHICH IS NECESSARY FOR SUBSEQUENT CALLS
C
C  FIRST CALL TO ODE --
C
C  THE USER MUST PROVIDE STORAGE IN HIS CALLING PROGRAM FOR THE ARRAYS
C  IN THE CALL LIST:
C     Y(NEQN), WORK(100+21*NEQN), IWORK(5),
C  DECLARE F IN AN EXTERNAL STATEMENT, SUPPLY THE SUBROUTINE
```

```
C   F(T,Y,YP) TO EVALUATE
C       DY(I)/DT = YP(I) = F(T,Y(1),Y(2),...,Y(NEQN))
C
C   ********  IMPORTANT NOTICE  ********
C   THE USER MUST DECLARE ALL THE REAL VARIABLES OF SUBROUTINE
C   F AS DOUBLE-PRECISION VARIABLES AND CONSEQUENTLY USE
C   FORTRAN DOUBLE-PRECISION BUILT-IN FUNCTIONS IF NECESSARY.
C   **********************************
C
C   AND INITIALIZE THE PARAMETERS:
C       NEQN -- NUMBER OF EQUATIONS TO BE INTEGRATED
C       Y(*) -- VECTOR OF INITIAL CONDITIONS
C       T -- STARTING POINT OF INTEGRATION
C       TOUT -- POINT AT WHICH SOLUTION IS DESIRED
C       RELERR,ABSERR -- RELATIVE AND ABSOLUTE LOCAL ERROR TOLERANCES
C       IFLAG -- +1,-1. INDICATOR TO INITIALIZE THE CODE. NORMAL INPUT
C             IS +1. THE USER SHOULD SET IFLAG=-1 ONLY IF IT IS
C             IMPOSSIBLE TO CONTINUE THE INTEGRATION BEYOND TOUT.
C   ALL PARAMETERS EXCEPT F, NEQN AND TOUT MAY BE ALTERED BY THE
C   CODE ON OUTPUT SO MUST BE VARIABLES IN THE CALLING PROGRAM.
C
C   OUTPUT FROM DE --
C
C       NEQN -- UNCHANGED
C       Y(*) -- SOLUTION AT T
C       T -- LAST POINT REACHED IN INTEGRATION. NORMAL RETURN HAS
C            T = TOUT.
C       TOUT -- UNCHANGED
C       RELERR,ABSERR -- NORMAL RETURN HAS TOLERANCES UNCHANGED. IFLAG=3
C            SIGNALS TOLERANCES INCREASED
C       IFLAG = 2 -- NORMAL RETURN INTEGRATION REACHED TOUT
C             = 3 -- INTEGRATION DID NOT REACH TOUT BECAUSE ERROR
C                    TOLERANCES TOO SMALL. RELERR, ABSERR INCREASED
C                    APPROPRIATELY FOR CONTINUING
C             = 4 -- INTEGRATION DID NOT REACH TOUT BECAUSE MORE THAN
C                    MAXNUM STEPS NEEDED
C             = 5 -- INTEGRATION DID NOT REACH TOUT BECAUSE EQUATIONS
C                    APPEAR TO BE STIFF
C             = 6 -- ATTEMPT TO COMPUTE A VANISHING SOLUTION WITH
C                    A PURE RELATIVE ERROR TEST.
C             = 7 -- INVALID INPUT PARAMETERS (FATAL ERROR)
C            THE VALUE OF IFLAG IS RETURNED NEGATIVE WHEN THE INPUT
C            VALUE IS NEGATIVE AND THE INTEGRATION DOES NOT REACH TOUT,
C            I.E., -3, -4, -5, -6.
C       WORK(*),IWORK(*) -- INFORMATION GENERALLY OF NO INTEREST TO
C              THE USER BUT NECESSARY FOR SUBSEQUENT CALLS.
C
C   SUBSEQUENT CALLS TO ODE --
C
C   SUBROUTINE ODE RETURNS WITH ALL INFORMATION NEEDED TO CONTINUE
C   THE INTEGRATION. IF THE INTEGRATION REACHED TOUT, THE USER NEED
C   ONLY DEFINE A NEW TOUT AND CALL AGAIN. IF THE INTEGRATION DID NOT
C   REACH TOUT AND THE USER WANTS TO CONTINUE, HE JUST CALLS AGAIN.
C   THE OUTPUT VALUE OF IFLAG IS THE APPROPRIATE INPUT VALUE FOR
C   SUBSEQUENT CALLS. THE ONLY SITUATION IN WHICH IT SHOULD BE ALTERED
C   IS TO STOP THE INTEGRATION INTERNALLY AT THE NEW TOUT, I.E.,
C   CHANGE OUTPUT IFLAG=2 TO INPUT IFLAG=-2. ERROR TOLERANCES MAY
C   BE CHANGED BY THE USER BEFORE CONTINUING. ALL OTHER PARAMETERS
C   MUST REMAIN UNCHANGED.
C
C*******************************************************************
C*  SUBROUTINES DE AND STEP CONTAIN MACHINE DEPENDENT CONSTANTS.   *
C*  BE SURE THEY ARE SET BEFORE USING ODE.                         *
C*******************************************************************
C
      IMPLICIT REAL*8 (A-H,O-Z)
      LOGICAL START,PHASE1,NORND
      DIMENSION Y(NEQN),WORK(1),IWORK(5)
      EXTERNAL F
      DATA IALPHA,IBETA,ISIG,IV,IW,IG,IPHASE,IPSI,IX,IH,IHOLD,
     @     ISTART,ITOLD,IDELSN/1,13,25,38,50,62,75,76,88,89,90,91,92,
     @     93/
      IYY=100
      IWT=IYY+NEQN
      IP=IWT+NEQN
      IYP=IP+NEQN
      IYPOUT=IYP+NEQN
      IPHI=IYPOUT+NEQN
      IF(IABS(IFLAG) .EQ. 1) GO TO 1
      START=WORK(ISTART) .GT. 0.0D0
      PHASE1=WORK(IPHASE) .GT. 0.0D0
      NORND=IWORK(2) .NE. -1
1     CALL DE(F,NEQN,Y,T,TOUT,RELERR,ABSERR,IFLAG,WORK(IYY),
     @  WORK(IWT),WORK(IP),WORK(IYP),WORK(IYPOUT),WORK(IPHI),
     @  WORK(IALPHA),WORK(IBETA),WORK(ISIG),WORK(IV),WORK(IW),
     @  WORK(IG),PHASE1,WORK(IPSI),WORK(IX),WORK(IH),WORK(IHOLD),
     @  START,WORK(ITOLD),WORK(IDELSN),IWORK(1),NORND,IWORK(3),
     @  IWORK(4),IWORK(5),ISTOP)
      IF (ISTOP .EQ. 1) RETURN
      WORK(ISTART)=-1.0D0
      IF(START) WORK(ISTART)=1.0D0
      WORK(IPHASE)=-1.0D0
      IF(PHASE1) WORK(IPHASE)=1.0D0
      IWORK(2)=-1
      IF(NORND) IWORK(2)=1
      RETURN
      END
C
C
C*******************************************************************
C
      SUBROUTINE DE(F,NEQN,Y,T,TOUT,RELERR,ABSERR,IFLAG,
     @  YY,WT,P,YP,YPOUT,PHI,ALPHA,BETA,SIG,V,W,G,PHASE1,PSI,X,H,
     @  HOLD,START,TOLD,DELSGN,NS,NORND,K,KOLD,ISNOLD,ISTOP)
C
C   ODE MERELY ALLOCATES STORAGE FOR DE TO RELIEVE THE USER OF THE
C   INCONVENIENCE OF A LONG CALL LIST. CONSEQUENTLY DE IS USED AS
C   DESCRIBED IN THE COMMENTS FOR ODE.
C
C   THIS CODE IS COMPLETELY EXPLAINED AND DOCUMENTED IN THE TEXT,
C   COMPUTER SOLUTION OF ORDINARY DIFFERENTIAL EQUATIONS: THE INITIAL
C   VALUE PROBLEM BY L.F. SHAMPINE AND M.K. GORDON, W.H. FREEMAN AND
C   COMPANY, SAN FRANCISCO, 1975.
C
      IMPLICIT REAL*8 (A-H,O-Z)
      LOGICAL STIFF,CRASH,START,PHASE1,NORND
      DIMENSION Y(NEQN),YY(NEQN),WT(NEQN),PHI(NEQN,16),P(NEQN),
     @  YP(NEQN),YPOUT(NEQN),PSI(12),ALPHA(12),BETA(12),SIG(13),
     @  V(12),W(12),G(13)
      EXTERNAL F
```

```
C
C*********************************************************************
C*  THE ONLY MACHINE DEPENDENT CONSTANT IS BASED ON THE MACHINE UNIT *
C*  ROUNDOFF ERROR U WHICH IS THE SMALLEST POSITIVE NUMBER SUCH THAT *
C*   1.0+U .GT. 1.0. U MUST BE CALCULATED AND FOURU=4.0*U INSERTED    *
C*   IN THE FOLLOWING DATA STATEMENT BEFORE USING DE. THE ROUTINE     *
C*  MACHIN CALCULATES U. FOURU AND TWOU=2.0*U MUST ALSO BE INSERTED   *
C*   IN SUBROUTINE STEP BEFORE CALLING DE.                            *
        DATA FOURU /0.8882D-15/                                        *
C*********************************************************************
C
C   THE CONSTANT MAXNUM IS THE MAXIMUM NUMBER OF STEPS ALLOWED IN ONE
C   CALL TO DE. THE USER MAY CHANGE THIS LIMIT BY ALTERING THE
C   FOLLOWING DATA STATEMENT
        DATA MAXNUM /500/
C
C            ***              ***                 ***
C   TEST FOR IMPROPER PARAMETERS
C
        IF(NEQN .LT. 1) GO TO 10
        IF(T .EQ. TOUT) GO TO 10
        IF(RELERR .LT. 0.0D0 .OR. ABSERR .LT. 0.0D0) GO TO 10
        EPS=DMAX1(RELERR,ABSERR)
        IF(EPS .LE. 0.0D0) GO TO 10
        IF(IFLAG .EQ. 0) GO TO 10
        ISN=ISIGN(1,IFLAG)
        IFLAG=IABS(IFLAG)
        IF(IFLAG .EQ. 1) GO TO 20
        IF(T .NE. TOLD) GO TO 10
        IF(IFLAG .GE. 2 .AND. IFLAG .LE. 5) GO TO 20
10      IFLAG=7
        RETURN
C
C   ON EACH CALL SET INTERVAL OF INTEGRATION AND COUNTER FOR NUMBER
C   OF STEPS. ADJUST INPUT TOLERANCES TO DEFINE WEIGHT VECTOR FOR
C   SUBROUTINE STEP
C
20      DEL=TOUT-T
        ABSDEL=DABS(DEL)
        TEND=T+10.0D0*DEL
        IF(ISN .LT  0) TEND=TOUT
        NOSTEP=0
        KLE4=0
        STIFF=.FALSE.
        RELEPS=RELERR/EPS
        ABSEPS=ABSERR/EPS
        IF(IFLAG .EQ. 1) GO TO 30
        IF(ISNOLD .LT. 0) GO TO 30
        IF(DELSGN*DEL  GT. 0.0D0) GO TO 50
C
C   ON START AND RESTART ALSO SET WORK VARIABLES X AND YY(*), STORE
C   THE DIRECTION OF INTEGRATION AND INITIALIZE THE STEP SIZE
C
30      START=.TRUE.
        X=T
        DO 40 L=1,NEQN
40      YY(L)=Y(L)
        DELSGN=DSIGN(1.0D0,DEL)
        H=DSIGN(DMAX1(DABS(TOUT-X),FOURU*DABS(X)),TOUT-X)
C
C   IF ALREADY PAST OUTPUT POINT, INTERPOLATE AND RETURN
C
50      IF(DABS(X-T) .LT. ABSDEL) GO TO 60
        CALL INTRP(X,YY,TOUT,Y,YPOUT,NEQN,KOLD,PHI,PSI)
        IFLAG=2
        T=TOUT
        TOLD=T
        ISNOLD=ISN
        RETURN
C
C   IF CANNOT GO PAST OUTPUT POINT AND SUFFICIENTLY CLOSE,
C   EXTRAPOLATE AND RETURN
C
60      IF(ISN .GT  0 .OR  DABS(TOUT-X) .GE  FOURU*DABS(X)) GO TO 80
        H=TOUT-X
        CALL F(X,YY,YP,ISTOP)
        IF (ISTOP .EQ. 1) RETURN
        DO 70 L=1,NEQN
70      Y(L)=YY(L)+H*YP(L)
        IFLAG=2
        T=TOUT
        TOLD=T
        ISNOLD=ISN
        RETURN
C
C   TEST FOR TOO MANY STEPS
C
80      IF(NOSTEP .LT. MAXNUM) GO TO 100
        IFLAG=ISN*4
        IF(STIFF) IFLAG=ISN*5
        DO 90 L=1,NEQN
90      Y(L)=YY(L)
        T=X
        TOLD=T
        ISNOLD=1
        RETURN
C
C   LIMIT STEP SIZE, SET WEIGHT VECTOR AND TAKE A STEP
C
100     H=DSIGN(DMIN1(DABS(H),DABS(TEND-X)),H)
        DO 110 L=1,NEQN
        WT(L)=RELEPS*DABS(YY(L))+ABSEPS
        IF(WT(L) .LE. 0.0D0) GO TO 140
110     CONTINUE
        CALL STEP(X,YY,F,NEQN,H,EPS,WT,START,
     @  HOLD,K,KOLD,CRASH,PHI,P,YP,PSI,
     @  ALPHA,BETA,SIG,V,W,G,PHASE1,NS,NORND,ISTOP)
         IF (ISTOP .EQ. 1) RETURN
C
C   TEST FOR TOLERANCES TOO SMALL
C
        IF(.NOT. CRASH) GO TO 120
        IFLAG=ISN*3
        RELERR=EPS*RELEPS
        ABSERR=EPS*ABSEPS
        DO 120 L=1,NEQN
120     Y(L)=YY(L)
        T=X
        TOLD=T
        ISNOLD=1
```

```
      RETURN
C
C  AUGMENT COUNTER ON NUMBER OF STEPS AND TEST FOR STIFFNESS
C
130   NOSTEP=NOSTEP+1
      KLE4=KLE4+1
      IF(KOLD .GT. 4) KLE4=0
      IF(KLE4 .GE. 50) STIFF=.TRUE.
      GO TO 50
140   IFLAG=ISN*5
      DO 150 L=1,NEON
150   Y(L)=YY(L)
      T=X
      TOLD=T
      ISNOLD=1
      RETURN
      END
C
C
C*******************************************************************
C
      SUBROUTINE STEP(X,Y,F,NEON,H,EPS,WT,START,
     &  HOLD,K,KOLD,CRASH,PHI,P,YP,PSI,
     &  ALPHA,BETA,SIG,V,W,G,PHASE1,NS,NORND,ISTOP)
C
C  SUBROUTINE STEP INTEGRATES A SYSTEM OF FIRST ORDER ORDINARY
C  DIFFERENTIAL EQUATIONS ONE STEP NORMALLY FROM X TO X+H, USING A
C  MODIFIED DIVIDED DIFFERENCE FORM OF THE ADAMS PECE FORMULAS. LOCAL
C  EXTRAPOLATION IS USED TO IMPROVE ABSOLUTE STABILITY AND ACCURACY.
C  THE CODE ADJUSTS ITS ORDER AND STEP SIZE TO CONTROL THE LOCAL ERROR
C  PER UNIT STEP IN A GENERALIZED SENSE. SPECIAL DEVICES ARE INCLUDED
C  TO CONTROL ROUNDOFF ERROR AND TO DETECT WHEN THE USER IS REQUESTING
C  TOO MUCH ACCURACY.
C
C  THIS CODE IS COMPLETELY EXPLAINED AND DOCUMENTED IN THE TEXT
C  COMPUTER SOLUTION OF ORDINARY DIFFERENTIAL EQUATIONS: THE INITIAL
C  VALUE PROBLEM BY L.F. SHAMPINE AND M.K. GORDON, W.H. FREEMAN AND
C  COMPANY, SAN FRANCISCO, 1975.
C
C
C  THE PARAMETERS REPRESENT:
C      X -- INDEPENDENT VARIABLE
C      Y(*) -- SOLUTION VECTOR AT X
C      YP(*) -- DERIVATIVE OF SOLUTION VECTOR AT X AFTER SUCCESSFUL
C           STEP
C      NEON -- NUMBER OF EQUATIONS TO BE INTEGRATED
C      H -- APPROPRIATE STEP SIZE FOR NEXT STEP. NORMALLY DETERMINED BY
C           CODE
C      EPS -- LOCAL ERROR TOLERANCE. MUST BE VARIABLE
C      WT(*) -- VECTOR OF WEIGHTS FOR ERROR CRITERION
C      START -- LOGICAL VARIABLE SET .TRUE. FOR FIRST STEP, .FALSE.
C           OTHERWISE
C      HOLD -- STEP SIZE USED FOR LAST SUCCESSFUL STEP
C      K -- APPROPRIATE ORDER FOR NEXT STEP (DETERMINED BY CODE)
C      KOLD -- ORDER USED FOR LAST SUCCESSFUL STEP
C      CRASH -- LOGICAL VARIABLE SET .TRUE. WHEN NO STEP CAN BE TAKEN,
C           .FALSE. OTHERWISE
C  THE ARRAYS PHI, PSI ARE REQUIRED FOR THE INTERPOLATION SUBROUTINE
C  INTRP. THE ARRAY P IS INTERNAL TO THE CODE.
C
C  INPUT TO STEP
C
C     FIRST CALL --
C
C  ****** NOTE ******
C  THIS VERSION OF STEP IS THE "STEP WITH THE FULL CALL LIST"
C  VERSION AS REFERRED TO BY THE AUTHORS. WITH THE EXCEPTION OF
C  PROVIDING STORAGE IN THE CALLING PROGRAM FOR
C    ALPHA(12),BETA(12),SIG(13),V(12),W(12),G(13)
C  AND DECLARING PHASE1 AND NORND AS LOGICAL VARIABLES, "STEP WITH
C  THE FULL CALL LIST" IS USED EXACTLY THE SAME WAY AS THE "BASIC
C  STEP". THIS CODE HAS BEEN EDITED TO PERFORM CALCULATIONS IN
C  DOUBLE-PRECISION (SEE NOTE IN ODE).
C  *****************
C
C  THE USER MUST PROVIDE STORAGE IN HIS DRIVER PROGRAM FOR ALL ARRAYS
C  IN THE CALL LIST, NAMELY
C
C    DIMENSION Y(NEON),WT(NEON),PHI(NEON,16),P(NEON),YP(NEON),PSI(12)
C
C  THE USER MUST ALSO DECLARE START AND CRASH LOGICAL VARIABLES
C  AND F AN EXTERNAL SUBROUTINE, SUPPLY THE SUBROUTINE F(X,Y,YP)
C  TO EVALUATE
C     DY(I)/DX = YP(I) = F(X,Y(1),Y(2),...,Y(NEON))
C  AND INITIALIZE ONLY THE FOLLOWING PARAMETERS:
C     X -- INITIAL VALUE OF THE INDEPENDENT VARIABLE
C     Y(*) -- VECTOR OF INITIAL VALUES OF DEPENDENT VARIABLES
C     NEON -- NUMBER OF EQUATIONS TO BE INTEGRATED
C     H -- NOMINAL STEP SIZE INDICATING DIRECTION OF INTEGRATION
C          AND MAXIMUM SIZE OF STEP. MUST BE VARIABLE
C     EPS -- LOCAL ERROR TOLERANCE PER STEP. MUST BE VARIABLE
C     WT(*) -- VECTOR OF NON-ZERO WEIGHTS FOR ERROR CRITERION
C     START -- .TRUE.
C
C  STEP REQUIRES THE L2 NORM OF THE VECTOR WITH COMPONENTS
C  LOCAL ERROR(L)/WT(L) BE LESS THAN EPS FOR A SUCCESSFUL STEP. THE
C  ARRAY WT ALLOWS THE USER TO SPECIFY AN ERROR TEST APPROPRIATE
C  FOR HIS PROBLEM. FOR EXAMPLE,
C     WT(L) = 1.0D0 SPECIFIES ABSOLUTE ERROR,
C           = DABS(Y(L)) ERROR RELATIVE TO THE MOST RECENT VALUE OF THE
C                L-TH COMPONENT OF THE SOLUTION,
C           = DABS(YP(L)) ERROR RELATIVE TO THE MOST RECENT VALUE OF THE
C                L-TH COMPONENT OF THE DERIVATIVE,
C           = DMAX1(WT(L),DABS(L))) ERROR RELATIVE TO THE LARGEST
C                MAGNITUDE OF L-TH COMPONENT OBTAINED SO FAR,
C           = DABS(Y(L))*RELERR/EPS + ABSERR/EPS SPECIFIES A MIXED
C               RELATIVE-ABSOLUTE TEST WHERE RELERR IS RELATIVE
C               ERROR, ABSERR IS ABSOLUTE ERROR AND EPS =
C               DMAX1(RELERR,ABSERR)
C
C     SUBSEQUENT CALLS --
C
C  SUBROUTINE STEP IS DESIGNED SO THAT ALL INFORMATION NEEDED TO
C  CONTINUE THE INTEGRATION, INCLUDING THE STEP SIZE H AND THE ORDER
C  K, IS RETURNED WITH EACH STEP. WITH THE EXCEPTION OF THE STEP
C  SIZE, THE ERROR TOLERANCE, AND THE WEIGHTS, NONE OF THE PARAMETERS
C  SHOULD BE ALTERED. THE ARRAY WT MUST BE UPDATED AFTER EACH STEP
C  TO MAINTAIN RELATIVE ERROR TESTS LIKE THOSE ABOVE. NORMALLY THE
C  INTEGRATION IS CONTINUED JUST BEYOND THE DESIRED ENDPOINT AND THE
C  SOLUTION INTERPOLATED THERE WITH SUBROUTINE INTRP. IF IT IS
```

```
C   IMPOSSIBLE TO INTEGRATE BEYOND THE END POINT, THE STEP SIZE MAY BE
C   REDUCED TO HIT THE ENDPOINT SINCE THE CODE WILL NOT TAKE A STEP
C   LARGER THAN H INPUT. CHANGING THE DIRECTION OF INTEGRATION
C   I.E., THE SIGN OF H, REQUIRES THE USER SET START = .TRUE. BEFORE
C   CALLING STEP AGAIN. THIS IS THE ONLY SITUATION IN WHICH START
C   SHOULD BE ALTERED.
C
C   OUTPUT FROM STEP
C
C      SUCCESSFUL STEP --
C
C   THE SUBROUTINE RETURNS AFTER EACH SUCCESSFUL STEP WITH START AND
C   CRASH SET .FALSE. . X REPRESENTS THE INDEPENDENT VARIABLE
C   ADVANCED ONE STEP OF LENGHT HOLD FROM ITS VALUE ON INPUT AND Y
C   THE SOLUTION VECTOR AT THE NEW VALUE OF X. ALL OTHER PARAMETERS
C   REPRESENT INFORMATION CORRESPONDING TO THE NEW X NEEDED TO
C   CONTINUE THE INTEGRATION.
C
C      UNSUCCESSFUL STEP --
C
C   WHEN THE ERROR TOLERANCE IS TOO SMALL FOR THE MACHINE PRECISION,
C   THE SUBROUTINE RETURNS WITHOUT TAKING A STEP AND CRASH = .TRUE.
C   AN APPROPRIATE STEP SIZE AND ERROR TOLERANCE FOR CONTINUING ARE
C   ESTIMATED AND ALL OTHER INFORMATION IS RESTORED AS UPON INPUT
C   BEFORE RETURNING. TO CONTINUE WITH THE LARGER TOLERANCE, THE USER
C   JUST CALLS THE CODE AGAIN. A RESTART IS NEITHER REQUIRED NOR
C   DESIRABLE.
C
      IMPLICIT REAL*8 (A-H,O-Z)
      LOGICAL START,CRASH,PHASE1,NORND
      DIMENSION Y(NEQN),WT(NEQN),PHI(NEQN,16),P(NEQN),YP(NEQN),PSI(12)
      DIMENSION ALPHA(12),BETA(12),SIG(13),W(12),V(12),G(13),
     @  GSTR(13),TWO(13)
      EXTERNAL F
C***********************************************************************
C*  THE ONLY MACHINE DEPENDENT CONSTANTS ARE BASED ON THE MACHINE UNIT *
C*  ROUNDOFF ERROR U WHICH IS THE SMALLEST POSITIVE NUMBER SUCH THAT   *
C*  1.0+U .GT. 1.0. THE USER MUST CALCULATE U AND INSERT TWOU=2.0*U    *
C*  AND FOURU=4.0*U IN THE DATA STATEMENT BEFORE CALLING THE CODE.     *
C*  THE ROUTINE MACHIN CALCULATES U
      DATA TWOU,FOURU /0.4441D-15,0.8882D-15/
C***********************************************************************
      DATA TWO/2.0D0,4.0D0,8.0D0,16.0D0,32.0D0,64.0D0,128.0D0,
     @  256.0D0,512.0D0,1024.0D0,2048.0D0,4096.0D0,8192.0D0/
      DATA GSTR/0.5000D0,0.0833D0,0.0417D0,0.0264D0,0.0188D0,0.0143D0,
     $  0.0144D0,0.00936D0,0.00789D0,0.00679D0,0.00592D0,
     @  0.00524D0,0.00468D0/
C
C
C       ***       BEGIN BLOCK 0      ***
C   CHECK IF STEP SIZE OR ERROR TOLERANCE IS TOO SMALL FOR MACHINE
C   PRECISION. IF FIRST STEP, INITIALIZE PHI ARRAY AND ESTIMATE A
C   STARTING STEP SIZE.
C                   ***
C
C   IF STEP SIZE IS TOO SMALL, DETERMINE AN ACCEPTABLE ONE
C
      CRASH=.TRUE.
      IF(DABS(H) .GE. FOURU*DABS(X)) GO TO 5
      H=DSIGN(FOURU*DABS(X),H)
      RETURN
 5    PSEPS=0.5D0*EPS
C
C   IF ERROR TOLERANCE IS TOO SMALL, INCREASE IT TO AN ACCEPTABLE VALUE
C
      ROUND=0.0D0
      DO 10 L=1,NEQN
 10   ROUND=ROUND+(Y(L)/WT(L))**2
      ROUND=TWOU*DSQRT(ROUND)
      IF(PSEPS .GE. ROUND) GO TO 15
      EPS=2.0D0*ROUND*(1.0D0+FOURU)
      RETURN
 15   CRASH=.FALSE.
      G(1)=1.0D0
      G(2)=0.5D0
      SIG(1)=1.0D0
      IF(.NOT. START) GO TO 99
C
C   INITIALIZE. COMPUTE APPROPRIATE STEP SIZE FOR FIRST STEP
C
      CALL F(X,Y,YP,ISTOP)
      IF (ISTOP .EQ. 1) RETURN
      SUM=0.0D0
      DO 20 L=1,NEQN
      PHI(L,1)=YP(L)
      PHI(L,2)=0.0D0
 20   SUM=SUM+(YP(L)/WT(L))**2
      SUM=DSQRT(SUM)
      ABSH=DABS(H)
      IF(EPS .LT. 16.0D0*SUM*H*H) ABSH=0.25D0*DSQRT(EPS/SUM)
      H=DSIGN(DMAX1(ABSH,FOURU*DABS(X)),H)
      HOLD=0.0D0
      K=1
      KOLD=0
      START=.FALSE.
      PHASE1=.TRUE.
      NORND=.TRUE.
      IF(PSEPS .GT. 100.0D0*ROUND) GO TO 99
      NORND=.FALSE.
      DO 25 L=1,NEQN
 25   PHI(L,15)=0.0D0
 99   IFAIL=0
C       ***     END BLOCK 0      ***
C
C       ***     BEGIN BLOCK 1    ***
C   COMPUTE COEFFICIENTS OF FORMULAS FOR THIS STEP. AVOID COMPUTING
C   THOSE QUANTITIES NOT CHANGED WHEN STEP SIZE IS NOT CHANGED.
C                   ***
C
 100  KP1=K+1
      KP2=K+2
      KM1=K-1
      KM2=K-2
C
C   NS IS THE NUMBER OF STEPS TAKEN WITH SIZE H, INCLUDING THE CURRENT
C   ONE. WHEN K .LT. NS, NO COEFFICIENTS CHANGE
C
      IF(H .NE. HOLD) NS=0
      NS=MIN0(NS+1,KOLD+1)
      NSP1=NS+1
      IF(K .LT. NS) GO TO 199
C
C  COMPUTE THOSE COMPONENTS OF ALPHA(*), BETA(*), PSI(*), SIG(*) WHICH
C  ARE CHANGED
C
      BETA(NS)=1.0D0
      REALNS=DFLOAT(NS)
      ALPHA(NS)=1.0D0/REALNS
      TEMP1=H*REALNS
      SIG(NSP1)=1.0D0
      IF(K .LT. NSP1) GO TO 110
      DO 105 I=NSP1,K
      IM1=I-1
      TEMP2=PSI(IM1)
      PSI(IM1)=TEMP1
      BETA(I)=BETA(IM1)*PSI(IM1)/TEMP2
      TEMP1=TEMP2+H
      ALPHA(I)=H/TEMP1
      REALI=DFLOAT(I)
105   SIG(I+1)=REALI*ALPHA(I)*SIG(I)
110   PSI(K)=TEMP1
C
C  COMPUTE COEFFICIENTS G(*)
C
C  INITIALIZE V(*) AND W(*).
C
      IF(NS .GT. 1) GO TO 120
      DO 115 IQ=1,K
      TEMP3=IQ*(IQ+1)
      V(IQ)=1.0D0/TEMP3
115   W(IQ)=V(IQ)
      GO TO 140
C
C  IF ORDER WAS RAISED, UPDATE DIAGONAL PART OF V(*)
C
120   IF(K .LE. KOLD) GO TO 130
      TEMP4=K*KP1
      V(K)=1.0D0/TEMP4
      NSM2=NS-2
      IF(NSM2 .LT. 1) GO TO 130
      DO 125 J=1,NSM2
      I=K-J
125   V(I)=V(I)-ALPHA(J+1)*V(I+1)
C
C  UPDATE V(*) AND SET W(*)
C
130   LIMIT1=KP1-NS
      TEMP5=ALPHA(NS)
      DO 135 IQ=1,LIMIT1
      V(IQ)=V(IQ)-TEMP5*V(IQ+1)
135   W(IQ)=V(IQ)
      G(NSP1)=W(1)
C
C  COMPUTE THE G(*) IN THE WORK VECTOR W(*)
C
140   NSP2=NS+2
      IF(KP1 .LT. NSP2) GO TO 199
      DO 150 I=NSP2,KP1
      LIMIT2=KP2-I
      TEMP6=ALPHA(I-1)
      DO 145 IQ=1,LIMIT2
145   W(IQ)=W(IQ)-TEMP6*W(IQ+1)
150   G(I)=W(1)
199   CONTINUE
C     ***     END BLOCK 1   ***
C
C     ***      BEGIN BLOCK 2      ***
C  PREDICT A SOLUTION P(*), EVALUATE DERIVATIVES USING PREDICTED
C  SOLUTION, ESTIMATE LOCAL ERROR AT ORDER K AND ERRORS AT ORDERS K,
C  K-1, K-2 AS IF CONSTANT STEP SIZE WERE USED.
C                    ***
C
C  CHANGE PHI TO PHI STAR
C
      IF(K .LT. NSP1) GO TO 215
      DO 210 I=NSP1,K
      TEMP1=BETA(I)
      DO 205 L=1,NEQN
205   PHI(L,I)=TEMP1*PHI(L,I)
210   CONTINUE
C
C  PREDICT SOLUTION AND DIFFERENCES
C
215   DO 220 L=1,NEQN
      PHI(L,KP2)=PHI(L,KP1)
      PHI(L,KP1)=0.0D0
220   P(L)=0.0D0
      DO 230 J=1,K
      I=KP1-J
      IP1=I+1
      TEMP2=G(I)
      DO 225 L=1,NEQN
      P(L)=P(L)+TEMP2*PHI(L,I)
225   PHI(L,I)=PHI(L,I)+PHI(L,IP1)
230   CONTINUE
      IF(NORND) GO TO 240
      DO 235 L=1,NEQN
      TAU=H*P(L)-PHI(L,15)
      P(L)=Y(L)+TAU
235   PHI(L,16)=(P(L)-Y(L))-TAU
      GO TO 250
240   DO 245 L=1,NEQN
245   P(L)=Y(L)+H*P(L)
250   XOLD=X
      X=X+H
      ABSH=DABS(H)
      CALL F(X,P,YP,ISTOP)
      IF (ISTOP .EQ. 1) RETURN
C
C  ESTIMATE ERRORS AT ORDERS K, K-1, K-2
C
      ERKM2=0.0D0
      ERKM1=0.0D0
      ERK=0.0D0
      DO 265 L=1,NEQN
      TEMP3=1.0D0/WT(L)
      TEMP4=YP(L)-PHI(L,1)
      IF(KM2) 265,260,255
255   ERKM2=ERKM2+((PHI(L,KM1)+TEMP4)*TEMP3)**2
260   ERKM1=ERKM1+((PHI(L,K)+TEMP4)*TEMP3)**2
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```
265    ERK=ERK+(TEMP4*TEMP3)**2
       IF(KM2) 280,275,270
270    ERKM2=ABSH*SIG(KM1)*GSTR(KM2)*DSQRT(ERKM2)
275    ERKM1=ABSH*SIG(K)*GSTR(KM1)*DSQRT(ERKM1)
280    TEMP5=ABSH*DSQRT(ERK)
       ERR=TEMP5*(G(K)-G(KP1))
       ERK=TEMP5*SIG(KP1)*GSTR(K)
       KNEW=K
C
C   TEST IF ORDER SHOULD BE LOWERED
C
       IF(KM2) 299,290,285
285    IF(DMAX1(ERKM1,ERKM2) .LE. ERK) KNEW=KM1
       GO TO 299
290    IF(ERKM1 .LE. 0.5D0*ERK) KNEW=KM1
C
C   TEST IF STEP SUCCESSFUL
C
299    IF(ERR .LE. EPS) GO TO 400
C         ***        END BLOCK 2      ***
C
C         ***        BEGIN BLOCK 3      ***
C   THE STEP IS UNSUCCESSFUL. RESTORE X, PHI(*,*), PSI(*).
C   IF THIRD CONSECUTIVE FAILURE, SET ORDER TO ONE. IF STEP FAILS MORE
C   THAN THREE TIMES, CONSIDER AN OPTIMAL STEP. DOUBLE ERROR
C   TOLERANCE AND RETURN IF ESTIMATED STEP SIZE IS TOO SMALL FOR MACHINE
C   PRECISION.
C                     ***
C
C   RESTORE X, PHI(*,*), AND PSI(*)
C
       PHASE1=.FALSE.
       X=XOLD
       DO 310 I=1,K
       TEMP1=1.0D0/BETA(I)
       IP1=I+1
       DO 305 L=1,NEQN
305    PHI(L,I)=TEMP1*(PHI(L,I)-PHI(L,IP1))
310    CONTINUE
       IF(K .LT. 2) GO TO 320
       DO 315 I=2,K
315    PSI(I-1)=PSI(I)-H
C
C   ON THIRD FAILURE, SET ORDER TO ONE. THEREAFTER, USE OPTIMAL STEP
C   SIZE
C
320    IFAIL=IFAIL+1
       TEMP2=0.5D0
       IF(IFAIL-3) 335,330,325
325    IF(P5EPS .LT. 0.25D0*ERK) TEMP2=DSQRT(P5EPS/ERK)
330    KNEW=1
335    H=TEMP2*H
       K=KNEW
       IF(DABS(H) .GE. FOURU*DABS(X)) GO TO 340
       CRASH=.TRUE.
       H=DSIGN(FOURU*DABS(X),H)
       EPS=EPS+EPS
       RETURN
340    GO TO 100
C         ***        END BLOCK 3      ***
C
C         ***        BEGIN BLOCK 4      ***
C   THE STEP IS SUCCESSFUL. CORRECT THE PREDICTED SOLUTION, EVALUATE
C   THE DERIVATIVES USING THE CORRECTED SOLUTION AND UPDATE THE
C   DIFFERENCES. DETERMINE BEST ORDER AND STEP SIZE FOR NEXT STEP.
C                     ***
C
400    KOLD=K
       HOLD=H
C
C   CORRECT AND EVALUATE
C
       TEMP1=H*G(KP1)
       IF(NORND) GO TO 410
       DO 405 L=1,NEQN
       RHO=TEMP1*(YP(L)-PHI(L,1))-PHI(L,16)
       Y(L)=P(L)+RHO
405    PHI(L,16)=(Y(L)-P(L))-RHO
       GO TO 420
410    DO 415 L=1,NEQN
415    Y(L)=P(L)+TEMP1*(YP(L)-PHI(L,1))
420    CALL F(X,Y,YP,ISTOP)
       IF (ISTOP .EQ. 1) RETURN
C
C   UPDATE DIFFERENCES FOR NEXT STEP
C
       DO 425 L=1,NEQN
       PHI(L,KP1)=YP(L)-PHI(L,1)
425    PHI(L,KP2)=PHI(L,KP1)-PHI(L,KP2)
       DO 435 I=1,K
       DO 430 L=1,NEQN
430    PHI(L,I)=PHI(L,I)+PHI(L,KP1)
435    CONTINUE
C
C   ESTIMATE ERROR AT ORDER K+1 UNLESS:
C     IN FIRST PHASE WHEN ALWAYS RAISE ORDER,
C     ALREADY DECIDED TO LOWER ORDER,
C     STEP SIZE NOT CONSTANT SO ESTIMATE UNRELIABLE
C
       ERKP1=0.0D0
       IF(KNEW .EQ. KM1 .OR. K .EQ. 12) PHASE1=.FALSE.
       IF(PHASE1) GO TO 450
       IF(KNEW .EQ. KM1) GO TO 455
       IF(KP1 .GT. NS) GO TO 460
       DO 440 L=1,NEQN
440    ERKP1=ERKP1+(PHI(L,KP2)/WT(L))**2
       ERKP1=ABSH*GSTR(KP1)*DSQRT(ERKP1)
C
C   USING ESTIMATED ERROR AT ORDER K+1, DETERMINE APPROPRIATE ORDER
C   FOR NEXT STEP
C
       IF(K .GT. 1) GO TO 445
       IF(ERKP1 .GE. 0.5D0*ERK) GO TO 460
       GO TO 450
445    IF(ERKM1 .LE. DMIN1(ERK,ERKP1)) GO TO 455
       IF(ERKP1 .GE. ERK .OR. K .EQ. 12) GO TO 460
C
C   HERE ERKP1 .LT. ERK .LT. DMAX1(ERKM1,ERKM2) ELSE ORDER WOULD HAVE
C   BEEN LOWERED IN BLOCK 2. THUS ORDER IS TO BE RAISED.
C
```

```
C  RAISE ORDER
C
450    K=KP1
       ERK=ERKP1
       GO TO 460
C
C  LOWER ORDER
C
455    K=KM1
       ERK=ERKM1
C
C  WITH NEW ORDER DETERMINE APPROPRIATE STEP SIZE FOR NEXT STEP
C
460    HNEW=H+H
       IF(PHASE1) GO TO 465
       IF(PSEPS .GE. ERK*TWO(K+1)) GO TO 465
       HNEW=H
       IF(PSEPS .GE. ERK) GO TO 465
       TEMP2=K+1
       R=(PSEPS/ERK)**(1.0D0/TEMP2)
       HNEW=ABSH*DMAX1(0.5D0,DMIN1(0.9D0,R))
       HNEW=DSIGN(DMAX1(HNEW,FOURU*DABS(X)),H)
465    H=HNEW
       RETURN
C        ***      END BLOCK 4      ***
       END
C
C
C*****************************************************************
C
       SUBROUTINE INTRP(X,Y,XOUT,YOUT,YPOUT,NEQN,KOLD,PHI,PSI)
C
C  THE METHODS IN SUBROUTINE STEP APPROXIMATE THE SOLUTION NEAR X
C  BY A POLYNOMIAL. SUBROUTINE INTRP APPROXIMATES THE SOLUTION AT
C  XOUT BY EVALUATING THE POLYNOMIAL THERE. INFORMATION DEFINING THIS
C  POLYNOMIAL IS PASSED FROM STEP SO INTRP CANNOT BE USED ALONE.
C
C  THIS CODE IS COMPLETELY EXPLAINED AND DOCUMENTED IN THE TEXT,
C  COMPUTER SOLUTION OF ORDINARY DIFFERENTIAL EQUATIONS: THE INITIAL
C  VALUE PROBLEM BY L F. SHAMPINE AND M.K. GORDON, W.H. FREEMAN AND
C  COMPANY, SAN FRANCISCO, 1975.
C
C  INPUT TO INTRP --
C
C  THE USER PROVIDE THE STORAGE IN THE CALLING PROGRAM FOR THE ARRAYS IN
C  THE CALL LIST
      IMPLICIT REAL*8 (A-H,O-Z)
      DIMENSION Y(NEQN),YOUT(NEQN),YPOUT(NEQN),PHI(NEQN,16),PSI(12)
C  AND DEFINES
C     XOUT -- POINT AT WHICH SOLUTION IS DESIRED.
C  THE REMAINING PARAMETERS ARE DEFINED IN STEP AND PASSED TO INTRP
C  FROM THAT SUBROUTINE
C
C  OUTPUT FROM INTRP --
C
C     YOUT(*) -- SOLUTION AT XOUT
C     YPOUT(*) -- DERIVATIVE OF SOLUTION AT XOUT
C  THE REMAINING PARAMETERS ARE RETURNED UNALTERED FROM THEIR INPUT
C  VALUES. INTEGRATION WITH STEP MAY BE CONTINUED.
C
      DIMENSION G(13),W(13),RHO(13)
      DATA G(1)/1.0D0/,RHO(1)/1.0D0/
C
      HI=XOUT-X
      KI=KOLD+1
      KIP1=KI+1
C
C  INITIALIZE W(*) FOR COMPUTING G(*)
C
      DO 5 I=1,KI
      TEMP1=I
5     W(I)=1.0D0/TEMP1
      TERM=0.0D0
C
C  COMPUTE G(*)
C
      DO 15 J=2,KI
      JM1=J-1
      PSIJM1=PSI(JM1)
      GAMMA=(HI+TERM)/PSIJM1
      ETA=HI/PSIJM1
      LIMIT1=KIP1-J
      DO 10 I=1,LIMIT1
10    W(I)=GAMMA*W(I)-ETA*W(I+1)
      G(J)=W(1)
      RHO(J)=GAMMA*RHO(JM1)
15    TERM=PSIJM1
C
C  INTERPOLATE
C
      DO 20 L=1,NEQN
      YPOUT(L)=0.0D0
20    YOUT(L)=0.0D0
      DO 30 J=1,KI
      I=KIP1-J
      TEMP2=G(I)
      TEMP3=RHO(I)
      DO 25 L=1,NEQN
      YOUT(L)=YOUT(L)+TEMP2*PHI(L,I)
25    YPOUT(L)=YPOUT(L)+TEMP3*PHI(L,I)
30    CONTINUE
      DO 35 L=1,NEQN
35    YOUT(L)=Y(L)+HI*YOUT(L)
      RETURN
      END
C
C
C***************************************************************
C
C                      UTILITY ROUTINES 2.
C
C***************************************************************
C
C  THE FOLLOWING ROUTINES ARE A SUITE OF CODES THAT CAN BE USED TO
C  COMPUTE THE INVERSE OF A SQUARE MATRIX OF ORDER N AND/OR SOLVE A
C  SYSTEM OF LINEAR EQUATIONS OF THE TYPE AX=B (WHERE X IS THE
C  SOLUTION VECTOR OF LENGHT N). THE ROUTINE GAUSS CAN ALSO BE USED
C  TO COMPUTE THE DETERMINANT OF A SQUARE MATRIX.
C
C  THE ALGORITHMS ARE BASED ON THE CODES DEVELOPED AND DESCRIBED
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C  BY FORSYTHE ET AL. AND FORSYTHE AND MOLER (SEE ROUTINE
C  DESCRIPTIONS FOR COMPLETE REFERENCES).
C
C  THE USER NEEDS ONLY TO CALL ROUTINE GAUSS WHICH IS IN FACT A
C  DRIVER ROUTINE THAT CONTROLS THE OVERALL COMPUTATION (SEE GAUSS
C  FOR DETAILS ON ITS USE).
C
C*****************************************************************
C
C       **********   LATEST VERSION MAY 3, 1983   **********
C                        BY CHRISTIAN TOUPIN.
C
C*****************************************************************
C
C  ROUTINE NAME  -GAUSS
C
C  PURPOSE  -IN PLACE INVERSE AND/OR EQUATION SOLUTION OR
C            DETERMINANT EVALUATION (MATRICES IN FULL STORAGE MODE).
C
C  USAGE  -CALL GAUSS(NDIM,N,IJOB,A,B,DT,IWORK,WORK,IER)
C
C  ARGUMENTS
C
C      NDIM -ROW DIMENSION OF A EXACTLY AS SPECIFIED IN THE DIMENSION
C            STATEMENT IN THE CALLING PROGRAM (INPUT).
C         N -ORDER OF A (INPUT).
C      IJOB -INPUT OPTION PARAMETER. IJOB=I IMPLIES:
C               IJOB=1  INVERT MATRIX A. A IS REPLACED BY ITS INVERSE
C               IJOB=2  SOLVE THE EQUATION AX=B. A IS REPLACED BY AN
C                       UPPER TRIANGULAR MATRIX U AND A PERMUTED
C                       VERSION OF A LOWER TRIANGULAR MATRIX I-L SO
C                       THAT (PERMUTATION MATRIX)*A=L*U.
C               IJOB=3  SOLVE AX=B AND INVERT MATRIX A. A IS REPLACED
C                       BY ITS INVERSE.
C               IJOB=4  COMPUTE THE DETERMINANT OF A. A IS REPLACED BY
C                       THE TRIANGULARIZED MATRIX.
C         A -INPUT/OUTPUT MATRIX OF DIMENSION N BY N (SEE PARAMETER
C            IJOB).
C         B -INPUT/OUTPUT VECTOR OF LENGHT N WHEN IJOB=2 OR 3.
C            OTHERWISE B IS NOT USED BUT MUST BE DECLARED AS A VECTOR
C            I.E., B(N) IN THE CALLING ROUTINE. ON INPUT, B CONTAINS
C            THE RIGHT HAND SIDE OF THE EQUATION AX=B. ON OUTPUT,
C            THE SOLUTION X REPLACES B.
C        DT -DETERMINANT OF MATRIX A WHEN IJOB=4 (OUTPUT). OTHERWISE
C            DT IS UNDEFINED.
C     IWORK -INTEGER WORK VECTOR OF LENGHT AT LEAST N. ITS INPUT
C            CONTENT IS IGNORED AND ITS OUTPUT CONTENT IS USUALLY
C            UNIMPORTANT AT THAT LEVEL. THE VECTOR MUST BE DECLARED
C            AND INCLUDED IN THE CALLING ROUTINE.
C      WORK -WORK VECTOR OF LENGHT AT LEAST N FOR IJOB=2 OR 4 AND
C            AT LEAST (N+1)*N FOR IJOB=1 OR 3. ITS INPUT CONTENT IS
C            IGNORED AND ITS OUTPUT CONTENT IS USUALLY UNIMPORTANT AT
C            THAT LEVEL. THE VECTOR MUST BE DECLARED AND INCLUDED IN
C            THE CALLING ROUTINE.
C       IER -ERROR PARAMETER (OUTPUT).
C              IER=1  INDICATES THAT IJOB WAS LESS THAN 1 OR GREATER
C                     THAN 4 (FATAL ERROR).
C              IER=2  INDICATES THAT MATRIX A IS SINGULAR TO WORKING
C                     PRECISION (FATAL ERROR).
C
C  PROGRAMMING NOTES
C
C       1. INPUT MATRIX A IS DESTROYED IN ALL CASES. INPUT VECTOR B
C          IS DESTROYED FOR IJOB=2 OR 3.
C
C       2. IER=2 IMPLIES THAT THE ESTIMATED CONDITION OF MATRIX A
C          IS SUCH THAT, TO WORKING PRECISION, A IS CONSIDERED
C          SINGULAR. THE MATRIX MAY OR MAY NOT BE MATHEMATICALLY
C          SINGULAR.
C
C       3. THE REQUESTED JOBS ARE ACTUALLY PERFORMED BY A SUITE OF
C          CODES: INVERT, LNSINV AND DET WHICH ARE BASED ON 2
C          ROUTINES: DECOMP AND SOLVE. GAUSS IS ESSENTIALLY A DRIVER
C          THAT CALLS FIRSTLY ROUTINE DECOMP TO DECOMPOSE THE INPUT
C          MATRIX A BY GAUSSIAN ELIMINATION AND TO ESTIMATE THE
C          CONDITION OF THE MATRIX. THEN, IF THE MATRIX IS NOT
C          CONSIDERED SINGULAR, GAUSS CALLS THE APPROPRIATE ROUTINE
C          AS SPECIFIED BY THE JOB OPTION PARAMETER.
C
C          ALL CALCULATIONS ARE PERFORMED IN DOUBLE-PRECISION
C          TO ENSURE MAXIMUM ACCURACY. THE INPUT/OUTPUT PARAMETERS
C          TO/FROM GAUSS MUST THEN BE TRANSFORMED TO DOUBLE-
C          PRECISION VARIABLES BEFORE CALLING GAUSS IF THE CALLING
C          ROUTINE DEALS WITH SINGLE-PRECISION REALS.
C
C  REMARKS
C
C    THE CODES OF DECOMP AND SOLVE ARE COMPLETELY EXPLAINED AND
C    DOCUMENTED IN THE TEXT: COMPUTER METHODS FOR MATHEMATICAL
C    COMPUTATIONS, BY G.E. FORSYTHE, M.A. MALCOLM AND C.B. MOLER,
C    PRENTICE-HALL SERIES IN AUTOMATIC COMPUTATION, PRENTICE-HALL
C    INC., ENGLEWOOD CLIFFS, 1977, CHAP. 3
C
C    NOTE: TO ENSURE MAXIMUM ACCURACY IN THE CALCULATIONS, THESE
C          ROUTINES ARE THE DOUBLE-PRECISION VERSIONS OF THE
C          ORIGINAL CODES.
C
C

      SUBROUTINE GAUSS(NDIM,N,IJOB,A,B,DT,IPVT,WORK,IER)
C
C
      INTEGER NDIM,N,IJOB,IER,IPVT
C
      DIMENSION A(NDIM,N),B(N),WORK(1),IPVT(N)
C
      DOUBLE PRECISION A,B,DT,WORK,COND,CONDP1
C
C
      IER=0
C
C  TEST FOR IMPROPER IJOB PARAMETER.
C
      IF((IJOB .GE. 1) .AND. (IJOB .LE. 4)) GO TO 10
      IER=1
      RETURN
10    CONTINUE
C
C  DECOMPOSE THE REAL MATRIX A BY GAUSSIAN ELIMINATION AND
C  ESTIMATE THE CONDITION OF THE MATRIX. THE ORIGINAL INPUT MATRIX
C  A IS DESTROYED AND REPLACED BY THE TRIANGULAR DECOMPOSITION OF A.
```

```
C
      CALL DECOMP(NDIM,N,A,COND,IPVT,WORK)
C
C  TEST FOR SINGULARITY. IF MATRIX SINGULAR GO BACK TO CALLING
C  ROUTINE.
C
      CONDP1=COND+1.0D0
      IF(.NOT.(CONDP1 .EQ. COND)) GO TO 20
      IER=2
      RETURN
20    CONTINUE
C
C  PERFORM THE JOB AS SPECIFIED BY INPUT OPTION PARAMETER IJOB.
C
      IF(IJOB .EQ. 1) CALL INVERT(NDIM,N,A,B,IPVT,WORK)
      IF((IJOB .EQ. 2) .OR. (IJOB .EQ. 3))
     *                     CALL LNSINV(NDIM,N,IJOB,A,B,IPVT,WORK)
      IF(IJOB .EQ. 4) CALL DET(NDIM,N,A,IPVT,DT)
      RETURN
      END
C
C
C**********************************************************************
C
C  ROUTINE NAME   -INVERT
C
C  PURPOSE   -USES THE TRIANGULARIZED MATRIX OBTAINED FROM ROUTINE
C             DECOMP AND STORED IN MATRIX A (SEE DECOMP), TO
C             COMPUTE THE INVERSE OF THE ORIGINAL INPUT MATRIX A
C             (INPUT PARAMETER OF GAUSS).
C
C  USAGE   -CALL INVERT(NDIM,N,A,B,IPVT,WORK)
C
C  ARGUMENTS  (SEE ROUTINE DECOMP AND GAUSS)
C
C  PROGRAMMING NOTES
C
C    WORK VECTOR IS USED HERE TO SAVE ELEMENTS OF THE INPUT VECTOR
C    B AND THE J-TH COLUMN ELEMENTS OF THE INVERSE MATRIX A, DURING
C    THE INVERSION PROCESS.
C
C    ON OUTPUT A CONTAINS THE INVERSE OF THE ORIGINAL MATRIX A
C    (INPUT PARAMETER OF GAUSS). VECTOR B CONTAINS THE UNALTERED
C    CONTENT OF INPUT VECTOR B.
C
C  REMARKS
C
C    THIS SUBROUTINE FOLLOWS THE ALGOL 60 PROCEDURE DESCRIBED IN:
C    COMPUTER SOLUTION OF LINEAR ALGEBRAIC SYSTEMS, BY G.E. FORSYTHE
C    AND C.B. MOLER, PRENTICE-HALL SERIES ON AUTOMATIC COMPUTATION,
C    PRENTICE-HALL INC., ENGLEWOOD CLIFFS, 1967, SECTION 18.
C
C    ALL CALCULATIONS ARE PERFORMED IN DOUBLE-PRECISION TO MINIMIZE
C    ROUND-OFF ERRORS.
C
C
       SUBROUTINE INVERT(NDIM,N,A,B,IPVT,WORK)
C
C

       INTEGER NDIM,N,IPVT,WCOUNT
C
       DIMENSION A(NDIM,N),B(N),IPVT(N),WORK(1)
C
       DOUBLE PRECISION A,B,WORK
C
C
C
C  SAVE CONTENT OF INPUT VECTOR B.
C
      DO 10 I=1,N
      WORK(I)=B(I)
10    CONTINUE
C
C  BUILD THE I-TH UNIT VECTOR, I.E., ALL COORDINATES ARE 0
C  EXCEPT FOR THE I-TH, WHICH IS 1.
C
      WCOUNT=N+1
      DO 20 J=1,N
      DO 30 I=1,N
      IF(I .EQ. J) B(I)=1.0D0
      IF(I .NE. J) B(I)=0.0D0
30    CONTINUE
      CALL SOLVE(NDIM,N,A,B,IPVT)
C
C  SAVE THE J-TH COLUMN ELEMENTS OF A-INVERSE.
C
      DO 40 I=1,N
      WORK(WCOUNT)=B(I)
      WCOUNT=WCOUNT+1
40    CONTINUE
20    CONTINUE
C
C  TRANSFER ALL ELEMENTS OF WORK BACK TO A AND B. ON OUTPUT A
C  CONTAINS THE INVERSE OF THE ORIGINAL MATRIX A AND B, ITS ORIGINAL
C  CONTENT.
C
      WCOUNT=N+1
      DO 50 J=1,N
      DO 50 I=1,N
      A(I,J)=WORK(WCOUNT)
      WCOUNT=WCOUNT+1
50    CONTINUE
      DO 60 I=1,N
      B(I)=WORK(I)
60    CONTINUE
      RETURN
      END
C
C
C**********************************************************************
C
C  ROUTINE NAME   -LNSINV
C
C  PURPOSE   -USES THE TRIANGULARIZED MATRIX OBTAINED FROM DECOMP
C             AND STORED IN A (SEE DECOMP), TO SOLVE THE EQUATION
C             AX=B. ON OUTPUT B CONTAINS THE SOLUTION VECTOR X. IF
C             IJOB=2, MATRIX A IS NOT ALTERED AND STILL CONTAINS
C             THE TRIANGULARIZED MATRIX. IF IJOB=3, MATRIX A CONTAINS
C             THE INVERSE OF THE ORIGINAL MATRIX A (INPUT PARAMETER
C             OF GAUSS).
```

```
C
C  USAGE   -CALL LNSINV(NDIM,N,IJOB,A,B,IPVT,WORK)
C
C  ARGUMENTS  (SEE ROUTINE DECOMP AND GAUSS)
C
C  REMARKS
C
C    THIS SUBROUTINE FOLLOWS THE SAMPLE PROGRAM DESCRIBED IN
C    FORSYTHE ET AL. (1977) CHAP. 3, SECTION 3.3 (SEE GAUSS).
C    ALL CALCULATIONS ARE PERFORMED IN DOUBLE-PRECISION TO MINIMIZE
C    ROUND-OFF ERRORS.
C
C
      SUBROUTINE LNSINV(NDIM,N,IJOB,A,B,IPVT,WORK)
C
C
      INTEGER NDIM,N,IJOB,IPVT
C
      DIMENSION A(NDIM,N),B(N),IPVT(N),WORK(1)
C
      DOUBLE PRECISION A,B,WORK
C
C  SOLVE SYSTEM AX=B.
C
      CALL SOLVE(NDIM,N,A,B,IPVT)
C
C  IF IJOB=3, COMPUTE THE INVERSE OF ORIGINAL MATRIX A.
C
      IF(IJOB .EQ. 2) RETURN
      CALL INVERT(NDIM,N,A,B,IPVT,WORK)
      RETURN
      END
C
C
C**************************************************************
C
C  ROUTINE NAME   -DET
C
C  PURPOSE   -USES THE TRIANGULARIZED MATRIX OBTAINED FROM DECOMP
C             AND STORED IN A (SEE DECOMP), TO CALCULATE THE
C             DETERMINANT OF THE ORIGINAL MATRIX A (INPUT PARAMETER
C             OF GAUSS).
C
C             ON OUTPUT A STILL CONTAINS THE TRIANGULARIZED MATRIX A.
C
C  USAGE   -CALL DET(NDIM,N,A,IPVT,DT)
C
C  ARGUMENTS  (SEE ROUTINE DECOMP AND GAUSS)
C
C  REMARKS
C
C    THIS SUBROUTINE USES THE MULTIPLICATION APPROACH AS DESCRIBED
C    BY FORSYTHE ET AL  (1977) CHAP. 3, SECTION 3.3 (SEE GAUSS) AND
C    FORSYTHE ET AL. (1967) SECTION 14 (SEE INVERT).
C
C    ALL CALCULATIONS ARE PERFORMED IN DOUBLE-PRECISION TO MINIMIZE
C    ROUND-OFF ERRORS.
C
C
      SUBROUTINE DET(NDIM,N,A,IPVT,DT)
C
C
      INTEGER NDIM,N,IPVT
C
      DIMENSION A(NDIM,N),IPVT(N)
C
      DOUBLE PRECISION A,DT,PROD
C
C  GET THE DETERMINANT OF ORIGINAL MATRIX A. SINCE SINGULARITY
C  HAS BEEN CHECKED AT AN EARLIER LEVEL (ROUTINE GAUSS), IT IS
C  ASSUMED THAT NO ELEMENTS ON THE DIAGONAL OF THE INPUT
C  TRIANGULARIZED MATRIX A ARE 0.
C
      PROD=1.0D0
      DO 10 I=1,N
      PROD=PROD*A(I,I)
10    CONTINUE
      DT=IPVT(N)*PROD
      RETURN
      END
C
C
C**************************************************************
C
C  THE ROUTINE DECOMP HAS BEEN SLIGHTLY MODIFIED TO MAKE IT FULLY
C  COMPATIBLE WITH DRIVER ROUTINE GAUSS. REFER TO THAT ROUTINE FOR
C  THE ARRAY DIMENSION SPECIFICATIONS.
C
      SUBROUTINE DECOMP(NDIM,N,A,COND,IPVT,WORK)
C
      INTEGER NDIM,N,IPVT,NM1,I,J,K,KP1,KB,KM1,M
      DIMENSION A(NDIM,N),WORK(1),IPVT(N)
      DOUBLE PRECISION A,WORK,COND,EK,T,ANORM,YNORM,ZNORM,DABS
C
C  DECOMPOSES A REAL MATRIX BY GAUSSIAN ELIMINATION AND
C  ESTIMATES THE CONDITION OF THE MATRIX.
C
C  USE ROUTINE SOLVE TO COMPUTE SOLUTIONS TO LINEAR SYSTEMS.
C
C  INPUT:
C
C    NDIM -DECLARED ROW DIMENSION OF THE ARRAY CONTAINING A.
C       N -ORDER OF THE MATRIX.
C       A -MATRIX TO BE TRIANGULARIZED.
C
C  OUTPUT:
C
C       A -CONTAINS AN UPPER TRIANGULAR MATRIX U AND A PERMUTED
C          VERSION OF A LOWER TRIANGULAR MATRIX I-L SO THAT
C          (PERMUTATION MATRIX)*A=L*U.
C    COND -AN ESTIMATE OF THE CONDITION OF A.
C          FOR THE LINEAR SYSTEM A*X=B, CHANGES IN A AND B MAY CAUSE
C          CHANGES COND TIMES AS LARGE IN X. IF COND+1.0 .EQ. COND,
C          A IS SINGULAR TO WORKING PRECISION. COND IS SET TO 1.0E+32
C          IF EXACT SINGULARITY IS DETECTED.
C    IPVT -THE PIVOT VECTOR.
C           IPVT(K)=THE INDEX OF THE K-TH PIVOT ROW
C           IPVT(N)=(-1)**(NUMBER OF INTERCHANGES)
C    WORK -THE VECTOR WORK MUST BE DECLARED AND INCLUDED IN THE CALL.
C          ITS INPUT CONTENT IS IGNORED. ITS OUTPUT CONTENT IS
```

```
C          USUALLY UNIMPORTANT.
C
C   THE DETERMINANT OF A CAN BE OBTAINED ON OUTPUT BY:
C        DET(A)=IPVT(N)*A(1,1)*A(2,2)*...*A(N,N).
C
C
      IPVT(N)=1
      IF(N .EQ. 1) GO TO 80
      NM1=N-1
C
C   COMPUTE 1-NORM OF A
C
      ANORM=0.0D0
      DO 10 J=1,N
      T=0.0D0
      DO 5 I=1,N
      T=T+DABS(A(I,J))
5     CONTINUE
      IF(T .GT. ANORM) ANORM=T
10    CONTINUE
C
C   GAUSSIAN ELIMINATION WITH PARTIAL PIVOTING.
C
      DO 35 K=1,NM1
      KP1=K+1
C
C   FIND PIVOT.
C
      M=K
      DO 15 I=KP1,N
      IF(DABS(A(I,K)) .GT. DABS(A(M,K))) M=I
15    CONTINUE
      IPVT(K)=M
      IF(M .NE. K) IPVT(N)=-IPVT(N)
      T=A(M,K)
      A(M,K)=A(K,K)
      A(K,K)=T
C
C   SKIP STEP IF PIVOT IS ZERO.
C
      IF(T .EQ. 0.0D0) GO TO 35
C
C   COMPUTE MULTIPLIERS.
C
      DO 20 I=KP1,N
      A(I,K)=-A(I,K)/T
20    CONTINUE
C
C   INTERCHANGE AND ELIMINATE BY COLUMNS
C
      DO 30 J=KP1,N
      T=A(M,J)
      A(M,J)=A(K,J)
      A(K,J)=T
      IF(T .EQ. 0.0D0) GO TO 30
      DO 25 I=KP1,N
      A(I,J)=A(I,J)+A(I,K)*T
25    CONTINUE
30    CONTINUE
35    CONTINUE
C
C   COND=(1-NORM OF A)*(AN ESTIMATE OF 1-NORM OF A-INVERSE)
C   ESTIMATE OBTAINED BY ONE STEP OF INVERSE ITERATION FOR THE
C   SMALL SINGULAR VECTOR. THIS INVOLVES SOLVING TWO SYSTEMS
C   OF EQUATIONS, (A-TRANSPOSE)*Y=E AND A*Z=Y WHERE E
C   IS A VECTOR OF +1 OR -1 CHOSEN TO CAUSE GROWTH IN Y.
C   ESTIMATE=(1-NORM OF Z)/(1-NORM OF Y).
C
C   SOLVE (A-TRANSPOSE)*Y=E.
C
      DO 50 K=1,N
      T=0.0D0
      IF(K .EQ. 1) GO TO 45
      KM1=K-1
      DO 40 I=1,KM1
      T=T+A(I,K)*WORK(I)
40    CONTINUE
45    EK=1.0D0
      IF(T .LT. 0.0D0) EK=-1.0D0
      IF(A(K,K) .EQ. 0.0D0) GO TO 90
      WORK(K)=-(EK+T)/A(K,K)
50    CONTINUE
      DO 60 KB=1,NM1
      K=N-KB
      T=0.0D0
      KP1=K+1
      DO 55 I=KP1,N
      T=T+A(I,K)*WORK(K)
55    CONTINUE
      WORK(K)=T
      M=IPVT(K)
      IF(M .EQ. K) GO TO 60
      T=WORK(M)
      WORK(M)=WORK(K)
      WORK(K)=T
60    CONTINUE
C
      YNORM=0.0D0
      DO 65 I=1,N
      YNORM=YNORM+DABS(WORK(I))
65    CONTINUE
C
C   SOLVE A*Z=Y.
C
      CALL SOLVE(NDIM,N,A,WORK,IPVT)
C
      ZNORM=0.0D0
      DO 70 I=1,N
      ZNORM=ZNORM+DABS(WORK(I))
70    CONTINUE
C
C   ESTIMATE CONDITION.
C
      COND=ANORM*ZNORM/YNORM
      IF(COND .LT. 1.0D0) COND=1.0D0
      RETURN
C
C   1-BY-1 INPUT MATRIX.
C
80    COND=1.0D0
```

```
      IF(A(1,1) .NE. 0.0D0) RETURN
C
C  EXACT SINGULARITY.
C
80    COND=1.0D+32
      RETURN
      END
C
C
C*********************************************************************
C
      SUBROUTINE SOLVE(NDIM,N,A,B,IPVT)
C
      INTEGER NDIM,N,IPVT,KB,KM1,NM1,KP1,I,K,M
      DIMENSION A(NDIM,N),B(N),IPVT(N)
      DOUBLE PRECISION A,B,T
C
C  SOLUTION OF LINEAR SYSTEM, A*X=B.
C
C  ********** IMPORTANT NOTICE **********
C  DO NOT USE IF ROUTINE DECOMP HAS DETECTED SINGULARITY.
C  **************************************
C
C  INPUT:
C
C    NDIM -DECLARED ROW DIMENSION OF ARRAY CONTAINING A.
C       N -ORDER OF MATRIX.
C       A -TRIANGULARIZED MATRIX OBTAINED FROM DECOMP.
C       B -RIGHT HAND SIDE VECTOR.
C    IPVT -PIVOT VECTOR OBTAINED FROM DECOMP.
C
C  OUTPUT:
C
C       B -SOLUTION VECTOR, X.
C
C
C
C  FORWARD ELIMINATION.
C
      IF(N .EQ. 1) GO TO 50
      NM1=N-1
      DO 20 K=1,NM1
      KP1=K+1
      M=IPVT(K)
      T=B(M)
      B(M)=B(K)
      B(K)=T
      DO 10 I=KP1,N
      B(I)=B(I)+A(I,K)*T
10    CONTINUE
20    CONTINUE
C
C  BACK SUBSTITUTION.
C
      DO 40 KB=1,NM1
      KM1=N-KB
      K=KM1+1
      B(K)=B(K)/A(K,K)
      T=-B(K)
      DO 30 I=1,KM1
      B(I)=B(I)+A(I,K)*T
30    CONTINUE
40    CONTINUE
50    B(1)=B(1)/A(1,1)
      RETURN
      END
C
C
C*********************************************************************
C
C                        UTILITY ROUTINES 3.
C
C*********************************************************************
C
C  THE FOLLOWING CODES ARE THREE MATRIX MULTIPLICATION ROUTINES
C  MULAB, MULABT AND MULATB PERFORMING THE FOLLOWING CALCULATIONS:
C
C                MULAB   - C = A * B
C               MULABT   - C = A * (B-TRANSPOSE)
C               MULATB   - C = (A-TRANSPOSE) * B
C
C  ALL ROUTINES ARE IN DOUBLE-PRECISION TO ENSURE MAXIMUM ACCURACY
C
C*********************************************************************
C
C       **********   LATEST VERSION MAY 1, 1983   **********
C                       BY CHRISTIAN TOUPIN.
C
C*********************************************************************
C
C  ROUTINE NAME  -MULAB
C
C  PURPOSE  -MATRIX MULTIPLICATION (FULL STORAGE MODE).
C
C  USAGE  -CALL MULAB(A,B,L,M,N,IA,IB,C,IC,IER)
C
C  ARGUMENTS
C
C      A -L BY M MATRIX STORED IN FULL STORAGE MODE (INPUT).
C      B -M BY N MATRIX STORED IN FULL STORAGE MODE (INPUT).
C      L -NUMBER OF ROWS IN A (INPUT).
C      M -NUMBER OF COLUMNS IN A AND ROWS IN B (INPUT).
C      N -NUMBER OF COLUMNS IN B (INPUT).
C     IA -ROW DIMENSION OF MATRIX A EXACTLY AS SPECIFIED IN THE
C         DIMENSION STATEMENT IN THE CALLING PROGRAM (INPUT).
C     IB -ROW DIMENSION OF MATRIX B EXACTLY AS SPECIFIED IN THE
C         DIMENSION STATEMENT IN THE CALLING PROGRAM (INPUT).
C      C -L BY N MATRIX CONTAINING THE PRODUCT C=A*B (OUTPUT).
C     IC -ROW DIMENSION OF MATRIX C EXACTLY AS SPECIFIED IN THE
C         DIMENSION STATEMENT IN THE CALLING PROGRAM (INPUT).
C    IER -ERROR PARAMETER (OUTPUT). IER=1 INDICATES A, B OR C
C         WAS DIMENSIONED INCORRECTLY (FATAL ERROR).
C
C  PROGRAMMING NOTES
C
C     THIS ROUTINE PERFORMS ALL CALCULATIONS IN DOUBLE-PRECISION
C     TO ENSURE MAXIMUM ACCURACY. THE INPUT/OUTPUT PARAMETERS
C     TO/FROM MULAB MUST THEN BE TRANSFORMED TO DOUBLE-PRECISION
C     VARIABLES BEFORE CALLING MULAB IF THE CALLING PROGRAM DEALS
C     WITH SINGLE-PRECISION REALS.
```

```
C
C
      SUBROUTINE MULAB(A,B,L,M,N,IA,IB,C,IC,IER)
C
C
      INTEGER L,M,N,IA,IB,IC,IER
C
      DIMENSION A(IA,M),B(IB,N),C(IC,N)
C
      DOUBLE PRECISION A,B,C
C
C
      IER=0
C
C  TEST FOR IMPROPER DIMENSIONALIZATION OF MATRICES.
C  IA, IB AND IC MUST BE .GE. L, M AND L RESPECTIVELY.
C
      IF((IA .GE. L) .AND. (IB .GE. M) .AND. (IC .GE. L)) GO TO 10
      IER=1
      RETURN
10    CONTINUE
C
C  MULTIPLY A AND B.
C
      DO 20 I=1,L
      DO 30 J=1,N
      C(I,J)=0.0D0
      DO 40 K=1,M
      C(I,J)=C(I,J)+(A(I,K)*B(K,J))
40    CONTINUE
30    CONTINUE
20    CONTINUE
      RETURN
      END
C
C
C*******************************************************************
C
C  ROUTINE NAME  -MULABT
C
C  PURPOSE  -MATRIX MULTIPLICATION OF MATRIX A BY THE TRANSPOSE OF
C            MATRIX B (FULL STORAGE MODE)
C
C  USAGE  -CALL MULABT(A,B,L,M,N,IA,IB,C,IC,IER)
C
C  ARGUMENTS
C
C      A -L BY M MATRIX STORED IN FULL STORAGE MODE (INPUT).
C      B -N BY M MATRIX STORED IN FULL STORAGE MODE (INPUT).
C      L -NUMBER OF ROWS IN A AND C (INPUT).
C      M -NUMBER OF COLUMNS IN A AND B (INPUT).
C      N -NUMBER OF ROWS IN B AND NUMBER OF COLUMNS IN C (INPUT).
C     IA -ROW DIMENSION OF MATRIX A EXACTLY AS SPECIFIED IN THE
C          DIMENSION STATEMENT IN THE CALLING PROGRAM (INPUT).
C     IB -ROW DIMENSION OF MATRIX B EXACTLY AS SPECIFIED IN THE
C          DIMENSION STATEMENT IN THE CALLING PROGRAM (INPUT).
C      C -L BY N MATRIX CONTAINING THE PRODUCT C=A*(B-TRANSPOSE)
C          (OUTPUT).
C     IC -ROW DIMENSION OF MATRIX C EXACTLY AS SPECIFIED IN THE
C          DIMENSION STATEMENT IN THE CALLING PROGRAM (INPUT).
C    IER -ERROR PARAMETER (OUTPUT). IER=1 INDICATES A, B OR C
C          WAS DIMENSIONED INCORRECTLY (FATAL ERROR).
C
C  PROGRAMMING NOTES
C
C      THIS ROUTINE PERFORMS ALL CALCULATIONS IN DOUBLE-PRECISION
C      TO ENSURE MAXIMUM ACCURACY. THE INPUT/OUTPUT PARAMETERS
C      TO/FROM MULABT MUST THEN BE TRANSFORMED TO DOUBLE-PRECISION
C      VARIABLES BEFORE CALLING MULABT IF THE CALLING PROGRAM DEALS
C      WITH SINGLE-PRECISION REALS.
C
C
      SUBROUTINE MULABT(A,B,L,M,N,IA,IB,C,IC,IER)
C
C
      INTEGER L,M,N,IA,IB,IC,IER
C
      DIMENSION A(IA,M),B(IB,N),C(IC,N)
C
      DOUBLE PRECISION A,B,C
C
C
      IER=0
C
C  TEST FOR IMPROPER DIMENSIONALIZATION OF MATRICES.
C  IA, IB AND IC MUST BE .GE. L, N AND L RESPECTIVELY.
C
      IF((IA .GE. L) .AND. (IB .GE. N) .AND. (IC .GE. L)) GO TO 10
      IER=1
      RETURN
10    CONTINUE
C
C  MULTIPLY A AND B-TRANSPOSE.
C
      DO 20 I=1,L
      DO 30 J=1,N
      C(I,J)=0.0D0
      DO 40 K=1,M
      C(I,J)=C(I,J)+(A(I,K)*B(J,K))
40    CONTINUE
30    CONTINUE
20    CONTINUE
      RETURN
      END
C
C
C*******************************************************************
C
C  ROUTINE NAME  -MULATB
C
C  PURPOSE  -MATRIX MULTIPLICATION OF THE TRANSPOSE OF MATRIX A BY
C            MATRIX B (FULL STORAGE MODE).
C
C  USAGE  -CALL MULATB(A,B,L,M,N,IA,IB,C,IC,IER)
C
C  ARGUMENTS
C
C      A -L BY M MATRIX STORED IN FULL STORAGE MODE (INPUT).
C      B -L BY N MATRIX STORED IN FULL STORAGE MODE (INPUT).
C      L -NUMBER OF ROWS IN A AND B (INPUT).
```

```
C       M -NUMBER OF COLUMNS IN A AND NUMBER OF ROWS IN C (INPUT).
C       N -NUMBER OF COLUMNS IN B AND C (INPUT).
C      IA -ROW DIMENSION OF MATRIX A EXACTLY AS SPECIFIED IN THE
C           DIMENSION STATEMENT IN THE CALLING PROGRAM (INPUT).
C      IB -ROW DIMENSION OF MATRIX B EXACTLY AS SPECIFIED IN THE
C           DIMENSION STATEMENT IN THE CALLING PROGRAM (INPUT).
C       C -M BY N MATRIX CONTAINING THE PRODUCT C=(A-TRANSPOSE)*B
C           (OUTPUT).
C      IC -ROW DIMENSION OF MATRIX C EXACTLY AS SPECIFIED IN THE
C           DIMENSION STATEMENT IN THE CALLING PROGRAM (INPUT).
C     IER -ERROR PARAMETER (OUTPUT). IER=1 INDICATES A, B OR C
C           WAS DIMENSIONED INCORRECTLY (FATAL ERROR).
C
C   PROGRAMMING NOTES
C
C       THIS ROUTINE PERFORMS ALL CALCULATIONS IN DOUBLE-PRECISION
C       TO ENSURE MAXIMUM ACCURACY. THE INPUT/OUTPUT PARAMETERS
C       TO/FROM MULATB MUST THEN BE TRANSFORMED TO DOUBLE-PRECISION
C       VARIABLES BEFORE CALLING MULATB IF THE CALLING PROGRAM DEALS
C       WITH SINGLE-PRECISION REALS.
C
C
        SUBROUTINE MULATB(A,B,L,M,N,IA,IB,C,IC,IER)
C
C
        INTEGER L,M,N,IA,IB,IC,IER
C
        DIMENSION A(IA,M),B(IB,N),C(IC,N)
C
        DOUBLE PRECISION A,B,C
C
C
        IER=0
C
C   TEST FOR IMPROPER DIMENSIONALIZATION OF MATRICES.
C   IA, IB AND IC MUST BE .GE. L, L AND M RESPECTIVELY.
C
        IF((IA .GE. L) .AND. (IB .GE. L) .AND. (IC .GE. M)) GO TO 10
        IER=1
        RETURN
10      CONTINUE
C
C   MULTIPLY A-TRANSPOSE AND B.
C
        DO 20 I=1,L
        DO 30 J=1,N
        C(I,J)=0.0D0
        DO 40 K=1,M
        C(I,J)=C(I,J)+(A(K,I)*B(K,J))
40      CONTINUE
30      CONTINUE
20      CONTINUE
        RETURN
        END
```

13.3 An example

We will present in the next two sections an example of the arrangement of a typical data file to be processed by the program MCPERM, along with the accompanying output. The data correspond to the transient behavior of human lymphocytes exposed to a 3M DMSO solution at 30°C.

13.3.1 Data file

The data necessary for each optimization run must be organized as follows. The reader is referred to the listing of subroutine INPUT (see section 13.2.2) for the complete definition of the variables. Note that in this example we used the CGS (Centimeter-Gram-Second) unit system instead of the SI (Système International) system. Note furthermore that the data are input in *FREE FORMAT* form.

```
8.319E07  30.0                          R  RFTEMP
1  30.0                                 TEMPL  (TEMPV(K), K = 1, TEMPL)
1  2  1                                 CONCL  SOLNUM  IMPNUM
18.016  70.84  211.0                    (PMVV(K), K = 1, SOLNUM+1)
0.003  2.817E-04                        (CSSOLM(K), K = 1, SOLNUM)
1.05E-05  2.10E-10  6.30                CAREAO  VCO  RNOV
1.00E-08  2.817E-04                     (CSIOV(K), K = 1, SOLNUM)
3                                       PCONUM
6.6056232E-14                           PMM(1,1) = PWW
2.6192785E-15                           PMM(2,1) = PS1W
1.6760203E-15                           PMM(3,1) = PS1S1
1                                       DATAFL
90                                      CASEM(1,1)
1.0000000   1.0000   00.0               (YV(K),  WTV(K),  XV(K),
0.7430167   1334.0   01.0                                 K = 1 , CASEM(1,1))
0.6145250   2235.0   02.0
0.6089384   1951.0   03.0
0.6201117   2573.0   04.0
0.6480445   2509.0   05.0
0.6648044   2562.0   06.0
0.6759775   2579.0   07.0
0.6871508   2575.0   08.0
0.6983239   2576.0   09.0
0.7094970   2442.0   10.0
0.7206703   2472.0   11.0
0.7262569   2379.0   12.0
0.7318434   2402.0   13.0
0.7430167   2325.0   14.0
0.7541899   2300.0   15.0
0.7597764   2300.0   16.0
0.7653630   2274.0   17.0
0.7709496   2222.0   18.0
0.7821228   2244.0   19.0
0.7932959   2198.0   20.0
0.7932959   2231.0   21.0
0.8044692   2187.0   22.0
0.8044692   2189.0   23.0
0.8156423   2160.0   24.0
0.8212289   2176.0   25.0
0.8212289   2182.0   26.0
0.8324022   2137.0   27.0
0.8379887   2113.0   28.0
0.8435753   2181.0   29.0
0.8491619   2110.0   30.0
0.8603351   2133.0   31.0
0.8547484   2087.0   32.0
0.8547484   2123.0   33.0
0.8659217   2124.0   34.0
0.8715083   2139.0   35.0
0.8770948   2120.0   36.0
0.8715083   2149.0   37.0
0.8715083   2102.0   38.0
0.8826814   2109.0   39.0
0.8938547   2096.0   40.0
0.8882681   2075.0   41.0
0.8994412   2080.0   42.0
0.9050278   2032.0   43.0
0.9050278   2011.0   44.0
0.9050278   2075.0   45.0
0.9106144   2091.0   46.0
0.9106144   2033.0   47.0
0.9217876   2018.0   48.0
0.9162011   2089.0   49.0
0.9162011   2071.0   50.0
0.9217876   2006.0   51.0
0.9273742   2013.0   52.0
0.9217876   2042.0   53.0
0.9329608   2018.0   54.0
0.9329608   1979.0   55.0
0.9329608   2000.0   56.0
0.9385473   1988.0   57.0
0.9385473   1980.0   58.0
0.9497206   2091.0   59.0
0.9385473   1957.0   60.0
0.9553072   1987.0   61.0
0.9497206   1955.0   62.0
0.9497206   2015.0   63.0
0.9497206   1958.0   64.0
0.9553072   2021.0   65.0
0.9664803   1992.0   66.0
0.9664803   1996.0   67.0
0.9664803   1928.0   68.0
0.9664803   1977.0   69.0
0.9720669   1978.0   70.0
0.9720669   1969.0   71.0
0.9720669   1976.0   72.0
0.9720669   1983.0   73.0
0.9608937   1974.0   74.0
0.9832401   1956.0   75.0
0.9776536   1904.0   76.0
0.9720669   1927.0   77.0
0.9720669   1948.0   78.0
0.9776536   1950.0   79.0
0.9776536   1968.0   80.0
0.9888267   2027.0   81.0
0.9888267   1917.0   82.0
0.9832401   1904.0   83.0
0.9776536   1940.0   84.0
0.9832401   1941.0   85.0
0.9720669   1928.0   86.0
0.9888267   1946.0   87.0
0.9832401   1919.0   88.0
0.9832401   1981.0   89.0
0.0  1.0E-08                            TOLABS  TOLREL
0.001  0.001  0  25                     TOLSPX  TOLAVE  TESTSF  ITEMAX
```

13.3.2 Output file

The output from program MCPERM (corresponding to the treatment of the previous data) is presented here. As can be seen, the output is self-explanatory and therefore does not need further comments.

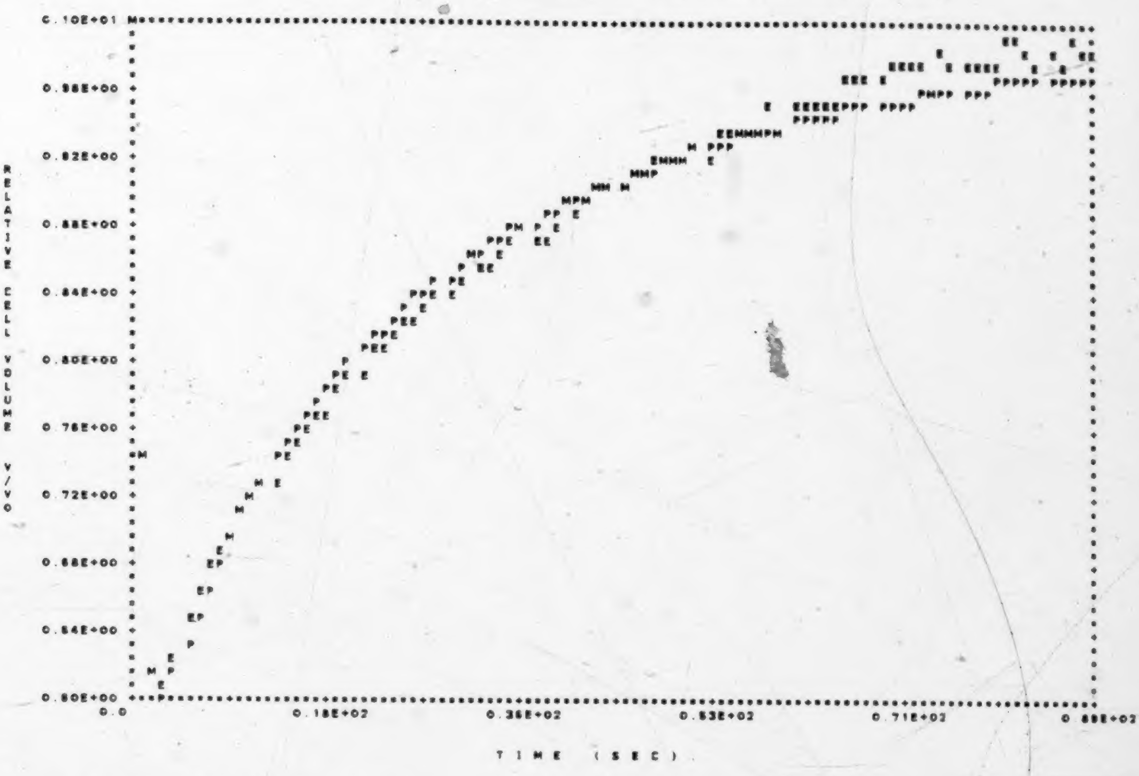
```
>>>>>>>  DATA INPUT.


UNIVERSAL GAS CONSTANT:  8.318E+07
REFERENCE TEMPERATURE:  30.0

TEMPERATURE(S) TO BE CONSIDERED.
 LEVEL INDEX     TEMPERATURE

      1               30.0

TOTAL NUMBER OF CONCENTRATION LEVELS (INCLUDING REPLICATES) TO BE CONSIDERED:   1
TOTAL NUMBER OF DIFFERENT SOLUTES:   2
TOTAL NUMBER OF IMPERMEABLE SOLUTES:   1

PARTIAL MOLAR VOLUME OF SOLVENT AND SOLUTES:

    VW        18.018
    VS(1)     70.940
    VS(2)    211.000

OSMOTICUM SOLUTE CONCENTRATIONS.
 LEVEL INDEX  SOLUTE INDEX     CONCENTRATION

      1             1             3.000E-03
      1             2             2.817E-04

INITIAL CELL MEMBRANE SURFACE AREA:  1.050E-06
INITIAL CELL VOLUME:  2.100E-10
RELATIVE NON-OSMOTIC VOLUME:    0.3000

INITIAL CELL SOLUTE CONCENTRATIONS.
 SOLUTE INDEX     CONCENTRATION

      1              1.000E-08
      2              2.817E-04

INITIAL GUESS OF CELL MEMBRANE MACROSCOPIC PERMEABILITY COEFFICIENTS.

 PERMEABILITY COEFFICIENT  APPARENT TRANSFER ENERGY

       6.807E-14
       2.819E-15
       1.678E-15


EXPERIMENTAL DATA SETS TO BE CONSIDERED.

*** VOLUME VALUES ARE GIVEN IN RELATIVE FORM ***

*** INITIAL VOLUMES ARE SET EXPLICITLY TO:  2.100E-10 ***


 TEMPERATURE LEVEL: 1       CONCENTRATION LEVEL: 1
 NUMBER OF CASES:   90

 CELL VOLUMES    RELATIVE CELL VOLUMES     ASSOCIATED WEIGHTS   TIME

   2.100E-10            1.0000                 1.000E+00         0.0
   1.560E-10            0.7430                 1.334E+03         1.00
   1.291E-10            0.6145                 2.236E+03         2.00
   1.279E-10            0.6089                 1.951E+03         3.00
   1.302E-10            0.6201                 2.673E+03         4.00
   1.361E-10            0.6480                 2.809E+03         5.00
   1.396E-10            0.6648                 2.662E+03         6.00
   1.420E-10            0.6760                 2.679E+03         7.00
   1.443E-10            0.6872                 2.576E+03         8.00
   1.466E-10            0.6983                 2.578E+03         9.00
   1.490E-10            0.7095                 2.442E+03        10.00
   1.513E-10            0.7207                 2.472E+03        11.00
   1.525E-10            0.7263                 2.378E+03        12.00
   1.537E-10            0.7318                 2.402E+03        13.00
   1.560E-10            0.7430                 2.326E+03        14.00
   1.584E-10            0.7542                 2.300E+03        15.00
   1.596E-10            0.7598                 2.300E+03        16.00
   1.607E-10            0.7654                 2.274E+03        17.00
   1.619E-10            0.7709                 2.222E+03        18.00
   1.642E-10            0.7821                 2.244E+03        19.00
   1.666E-10            0.7933                 2.198E+03        20.00
   1.666E-10            0.7933                 2.231E+03        21.00
   1.689E-10            0.8045                 2.197E+03        22.00
   1.689E-10            0.8045                 2.189E+03        23.00
   1.713E-10            0.8155                 2.160E+03        24.00
   1.725E-10            0.8212                 2.176E+03        25.00
   1.725E-10            0.8212                 2.192E+03        26.00
   1.748E-10            0.8324                 2.137E+03        27.00
   1.760E-10            0.8380                 2.113E+03        28.00
   1.772E-10            0.8436                 2.181E+03        29.00
   1.783E-10            0.8492                 2.110E+03        30.00
   1.807E-10            0.8603                 2.133E+03        31.00
   1.795E-10            0.8547                 2.087E+03        32.00
   1.795E-10            0.8547                 2.123E+03        33.00
   1.818E-10            0.8659                 2.124E+03        34.00
   1.830E-10            0.8715                 2.139E+03        35.00
   1.842E-10            0.8771                 2.120E+03        36.00
   1.830E-10            0.8715                 2.149E+03        37.00
   1.830E-10            0.8715                 2.102E+03        38.00
   1.854E-10            0.8827                 2.109E+03        39.00
   1.877E-10            0.8939                 2.089E+03        40.00
   1.865E-10            0.8883                 2.075E+03        41.00
   1.889E-10            0.8994                 2.080E+03        42.00
   1.901E-10            0.9050                 2.032E+03        43.00
   1.901E-10            0.9050                 2.011E+03        44.00
   1.901E-10            0.9050                 2.075E+03        45.00
   1.912E-10            0.9106                 2.081E+03        46.00
   1.912E-10            0.9106                 2.033E+03        47.00
   1.936E-10            0.9218                 2.018E+03        48.00
   1.924E-10            0.9162                 2.089E+03        49.00
```

```
1.924E-10          0.9162          2.071E+03     50.00
1.936E-10          0.9218          2.005E+03     51.00
1.947E-10          0.9274          2.013E+03     52.00
1.936E-10          0.9218          2.042E+03     53.00
1.959E-10          0.9330          2.018E+03     54.00
1.959E-10          0.9330          1.978E+03     55.00
1.959E-10          0.9330          2.000E+03     56.00
1.971E-10          0.9385          1.968E+03     57.00
1.971E-10          0.9385          1.980E+03     58.00
1.994E-10          0.9497          2.091E+03     59.00
1.971E-10          0.9385          1.957E+03     60.00
2.006E-10          0.9553          1.987E+03     61.00
1.994E-10          0.9497          1.965E+03     62.00
1.994E-10          0.9497          2.015E+03     63.00
1.994E-10          0.9497          1.968E+03     64.00
2.006E-10          0.9553          2.021E+03     65.00
2.030E-10          0.9665          1.982E+03     66.00
2.030E-10          0.9665          1.986E+03     67.00
2.030E-10          0.9665          1.929E+03     68.00
2.030E-10          0.9665          1.977E+03     69.00
2.041E-10          0.9721          1.978E+03     70.00
2.041E-10          0.9721          1.989E+03     71.00
2.041E-10          0.9721          1.978E+03     72.00
2.041E-10          0.9721          1.983E+03     73.00
2.018E-10          0.9609          1.974E+03     74.00
2.065E-10          0.9832          1.955E+03     75.00
2.053E-10          0.9777          1.904E+03     76.00
2.041E-10          0.9721          1.927E+03     77.00
2.041E-10          0.9721          1.948E+03     78.00
2.053E-10          0.9777          1.950E+03     79.00
2.053E-10          0.9777          1.969E+03     80.00
2.077E-10          0.9888          2.027E+03     81.00
2.077E-10          0.9888          1.917E+03     82.00
2.065E-10          0.9832          1.904E+03     83.00
2.053E-10          0.9777          1.940E+03     84.00
2.065E-10          0.9832          1.941E+03     85.00
2.041E-10          0.9721          1.928E+03     86.00
2.077E-10          0.9888          1.948E+03     87.00
2.065E-10          0.9832          1.919E+03     88.00
2.065E-10          0.9832          1.951E+03     89.00


TOLERANCES FOR ERROR CONTROL IN ADAM'S ALGORITHM.

 ABSOLUTE ERROR TEST:  0.0
 RELATIVE ERROR TEST:  1.000E-06


TEST VALUES FOR HALTING FIRST SIMPLEX SEARCH.

 COEFFICIENT OF VARIATION:  1.000E-03
 RELATIVE DIFFERENCE BETWEEN AVERAGES:  1.000E-03


*** NO LISTING OF TEST VARIABLES ***


*** A MAXIMUM OF    25 ITERATIONS IS ALLOWED IN ANY SIMPLEX SEARCH ***


NUMBER OF PARAMETERS TO OPTIMIZE:    3

INITIAL COEFFICIENTS IN PARAMETER MATRIX.

  ROW ( 1)       5.5066E-14
  ROW ( 2)       2.6193E-15
  ROW ( 3)       1.6760E-15
```

```
>>>>>>  SIMPLEX OPTIMIZATION IN PROGRESS.


  ITERATION NUMBER:      1
  CHI-SQUARE VALUE AT CURRENT BEST VERTEX:  2.166E+01
  PARAMETER VALUES:

     P( 1)       5.5066E-14
     P( 2)       2.6193E-15
     P( 3)       1.6760E-15

  ITERATION NUMBER:      2
  CHI-SQUARE VALUE AT CURRENT BEST VERTEX:  2.166E+01
  PARAMETER VALUES:

     P( 1)       5.5066E-14
     P( 2)       2.6193E-15
     P( 3)       1.6760E-15

  ITERATION NUMBER:      3
  CHI-SQUARE VALUE AT CURRENT BEST VERTEX:  2.166E+01
  PARAMETER VALUES:

     P( 1)       5.5066E-14
     P( 2)       2.6193E-15
     P( 3)       1.6760E-15

  ITERATION NUMBER:      4
  CHI-SQUARE VALUE AT CURRENT BEST VERTEX:  2.166E+01
  PARAMETER VALUES:

     P( 1)       5.5066E-14
     P( 2)       2.6193E-15
     P( 3)       1.6760E-15

  ITERATION NUMBER:      5
  CHI-SQUARE VALUE AT CURRENT BEST VERTEX:  2.166E+01
  PARAMETER VALUES:

     P( 1)       5.5066E-14
     P( 2)       2.6193E-15
     P( 3)        1.6760E-15

   ITERATION NUMBER:       5
   CHI-SQUARE VALUE AT CURRENT BEST VERTEX:  2.168E+01
   PARAMETER VALUES:

     P( 1)        6.6066E-14
     P( 2)        2.8193E-15
     P( 3)        1.6760E-15

   ***** OPTIMUM VERTEX FOUND. *****

     P( 1)        6.6066E-14
     P( 2)        2.8193E-15
     P( 3)        1.6760E-15

 CHI-SQUARE VALUE AT OPTIMUM VERTEX:  2.168E+01

       5 ITERATION(S).


 MAXIMUM RELATIVE ERROR IN CHI-SQUARE CALCULATIONS:  4.766E-07
 LIMIT OF EXPANSION OF OPTIMUM SIMPLEX:  4.766E-04

 EXPANSION OF SIMPLEX IN PROGRESS. VERTEX:  4

 MAXIMUM NUMBER OF SIMPLEX SEARCHES STILL ALLOWED:   3

 >>>>>>  QUADRATIC APPROXIMATION IN PROGRESS.

 >>>>>>  MINORS OF THE HESSIAN MATRIX ARE BEING CHECKED.

 VARIANCE-COVARIANCE MATRIX OF ESTIMATES

    2.6081E-30    5.7879E-32    1.1505E-32
    5.7879E-32    1.1272E-31   -4.5756E-33
    1.1505E-32   -4.5756E-33    5.2340E-34

                 PARAMETERS          STANDARD ERRORS        PERCENT ERRORS

      P( 1)      6.6402E-14            1.6150E-15                2.43
      P( 2)      2.8106E-15            3.3574E-16               11.94
      P( 3)      1.6824E-15            2.2878E-17                1.36

 CHI-SQUARE VALUE AT THE OPTIMUM:  2.166E+01

 NUMBER OF COMPLETE SIMPLEX SEARCHES:     1

 ______________________________________________________________________________________

 >>>>>>  TABLES OF PREDICTED VS OBSERVED RELATIVE CELL VOLUMES.



 TEMPERATURE OF SYSTEM:  30.0

 SOLUTE CONCENTRATIONS IN OSMOTICUM.
  SOLUTE INDEX    CONCENTRATION

       1             3.000E-03
       2             2.917E-04

   TIME      OBSERVED          PREDICTED          RESIDUALS          WEIGHTS

   0.0        1.0000            1.0000           0.0              1.000E+00
   1.00       0.7430            0.7435          -4.780E-04        1.334E+03
   2.00       0.6145            0.6179          -3.380E-03        2.238E+03
   3.00       0.6089            0.5998           9.104E-03        1.951E+03
   4.00       0.6201            0.6142           5.844E-03        2.673E+03
   5.00       0.6480            0.6328           1.523E-02        2.609E+03
   6.00       0.6648            0.6507           1.415E-02        2.682E+03
   7.00       0.6760            0.6671           8.924E-03        2.679E+03
   8.00       0.6872            0.6821           5.083E-03        2.576E+03
   9.00       0.6883            0.6859           2.389E-03        2.576E+03
  10.00       0.7095            0.7088           7.450E-04        2.442E+03
  11.00       0.7207            0.7207          -9.353E-07        2.472E+03
  12.00       0.7263            0.7318          -5.543E-03        2.379E+03
  13.00       0.7318            0.7422          -1.038E-02        2.402E+03
  14.00       0.7430            0.7520          -9.010E-03        2.325E+03
  15.00       0.7542            0.7613          -7.071E-03        2.300E+03
  16.00       0.7598            0.7700          -1.021E-02        2.300E+03
  17.00       0.7654            0.7782          -1.286E-02        2.274E+03
  18.00       0.7709            0.7861          -1.512E-02        2.222E+03
  19.00       0.7821            0.7935          -1.138E-02        2.244E+03
  20.00       0.7933            0.8006          -7.284E-03        2.198E+03
  21.00       0.7933            0.8073          -1.402E-02        2.231E+03
  22.00       0.8045            0.8138          -9.281E-03        2.197E+03
  23.00       0.8045            0.8199          -1.542E-02        2.189E+03
  24.00       0.8156            0.8258          -1.012E-02        2.160E+03
  25.00       0.8212            0.8314          -1.018E-02        2.176E+03
  26.00       0.8212            0.8368          -1.564E-02        2.182E+03
  27.00       0.8324            0.8419          -9.528E-03        2.137E+03
  28.00       0.8380            0.8469          -8.891E-03        2.113E+03
  29.00       0.8436            0.8516          -8.060E-03        2.161E+03
  30.00       0.8492            0.8562          -7.043E-03        2.110E+03
  31.00       0.8603            0.8606          -2.634E-04        2.133E+03
  32.00       0.8547            0.8648          -1.008E-02        2.087E+03
  33.00       0.8547            0.8689          -1.418E-02        2.123E+03
  34.00       0.8659            0.8728          -6.894E-03        2.124E+03
  35.00       0.8715            0.8766          -5.085E-03        2.139E+03
  36.00       0.8771            0.8802          -3.140E-03        2.120E+03
  37.00       0.8715            0.8837          -1.224E-02        2.148E+03
  38.00       0.8715            0.8871          -1.563E-02        2.102E+03
  39.00       0.8827            0.8904          -7.731E-03        2.109E+03
  40.00       0.8939            0.8936           2.615E-04        2.098E+03
  41.00       0.8883            0.8966          -8.359E-03        2.075E+03
  42.00       0.8994            0.8996          -1.381E-04        2.060E+03
  43.00       0.9050            0.9024           2.596E-03        2.032E+03
```

```
44.00      0.9050      0.9052      -1.555E-04    2.011E+03
45.00      0.9050      0.9079      -2.838E-03    2.075E+03
46.00      0.9106      0.9104       1.570E-04    2.091E+03
47.00      0.9106      0.9129      -2.334E-03    2.033E+03
48.00      0.9218      0.9154       6.417E-03    2.018E+03
49.00      0.9162      0.9177      -1.515E-03    2.089E+03
50.00      0.9162      0.9200      -3.787E-03    2.071E+03
51.00      0.9218      0.9222      -4.014E-04    2.005E+03
52.00      0.9274      0.9243       3.082E-03    2.013E+03
53.00      0.9218      0.9264      -4.603E-03    2.042E+03
54.00      0.9330      0.9284       4.565E-03    2.016E+03
55.00      0.9330      0.9303       2.621E-03    1.979E+03
56.00      0.9330      0.9322       7.346E-04    2.000E+03
57.00      0.9385      0.9341       4.491E-03    1.986E+03
58.00      0.9385      0.9358       2.718E-03    1.980E+03
59.00      0.9497      0.9376       1.217E-02    2.091E+03
60.00      0.9385      0.9392      -6.788E-04    1.957E+03
61.00      0.9553      0.9408       1.448E-02    1.987E+03
62.00      0.9497      0.9424       7.285E-03    1.965E+03
63.00      0.9497      0.9440       5.765E-03    2.015E+03
64.00      0.9497      0.9454       4.279E-03    1.968E+03
65.00      0.9553      0.9469       8.422E-03    2.021E+03
66.00      0.9665      0.9483       1.819E-02    1.982E+03
67.00      0.9665      0.9497       1.683E-02    1.996E+03
68.00      0.9665      0.9510       1.551E-02    1.929E+03
69.00      0.9665      0.9523       1.422E-02    1.977E+03
70.00      0.9721      0.9535       1.856E-02    1.976E+03
71.00      0.9721      0.9547       1.734E-02    1.969E+03
72.00      0.9721      0.9559       1.618E-02    1.978E+03
73.00      0.9721      0.9571       1.501E-02    1.983E+03
74.00      0.9609      0.9582       2.721E-03    1.974E+03
75.00      0.9832      0.9593       2.398E-02    1.958E+03
76.00      0.9777      0.9603       1.734E-02    1.904E+03
77.00      0.9721      0.9613       1.073E-02    1.927E+03
78.00      0.9721      0.9623       9.728E-03    1.948E+03
79.00      0.9777      0.9633       1.434E-02    1.950E+03
80.00      0.9777      0.9643       1.340E-02    1.969E+03
81.00      0.9888      0.9652       2.365E-02    2.027E+03
82.00      0.9888      0.9661       2.275E-02    1.917E+03
83.00      0.9832      0.9669       1.630E-02    1.904E+03
84.00      0.9777      0.9678       9.864E-03    1.940E+03
85.00      0.9832      0.9686       1.463E-02    1.941E+03
86.00      0.9721      0.9694       2.650E-03    1.928E+03
87.00      0.9888      0.9702       1.863E-02    1.948E+03
88.00      0.9832      0.9710       1.226E-02    1.919E+03
89.00      0.9832      0.9717       1.154E-02    1.981E+03

CALCULATED CHI-SQUARE AT OPTIMUM:  2.195E+01
```

PLOT OF VOLUME CHANGE OF STUDIED CELLS: EXPERIMENTAL (E) PREDICTED (P).



TOTAL SUM OF SQUARES FOR ALL SETS: 2.185E+01

14. APPENDIX 3: LISTING OF PROGRAMS *DATATR* AND *WMEAN*

14.1 General description of the programs

The following programs have been written in *FORTRAN 77* as implemented for the compiler developed by IBM Corporation (Anonymous, 1983). The reader is therefore referred to that reference for details concerning the statements appearing in the codes.

Programs DATATR and WMEAN are used basically to transform experimental data obtained from kinetic studies of volume changes of cellular suspensions (using an electronic particle counter) in a form suitable for treatment by the program MCPERM. Briefly, DATATR estimates the variances of the experimental data and generates, according to the relations disclosed in section 4.3.3, the "weights" to be used by the program MCPERM for the optimization search. DATATR also transforms the digitized readouts from the counter to cell volume values using the instrument calibration factor and the known volume of the calibration beads (see section 4.4). Finally, DATATR estimates, from these transformed values, the initial corpuscular volume and the initial volume change of the suspension.

The program WMEAN, for its part, is used to estimate the weighted grand mean, its variance and the variance of the parent population of individuals of a sample of means taken from that population (see section 4.3.3). This program is employed mostly for analyzing data coming from calibration and isotonic studies where the measurements are not a function of time (see section 4.4).

```
************************************************************************
*                                                                      *
*  PROGRAM NAME  - DATATR                                              *
*                                                                      *
*  LATEST VERSION - MARCH 20, 1985.                                    *
*                                                                      *
*  WRITTEN BY  - CHRISTIAN TOUPIN                                      *
*                FOOD ENGINEERING GROUP                                *
*                DEPARTMENT OF FOOD SCIENCE                            *
*                UNIVERSITY OF ALBERTA                                 *
*                EDMONTON, ALBERTA.                                    *
*                                                                      *
*  PURPOSE  - THIS PROGRAM IS USED TO TRANSFORM THE EXPERIMENTAL       *
*             DATA OBTAINED FROM KINETIC STUDIES OF VOLUME CHANGES     *
*             OF CELLULAR SUSPENSIONS (USING AN ELECTRONIC             *
*             PARTICLE COUNTER) IN A FORM SUITABLE FOR THE             *
*             ESTIMATION OF THE CELLS' MACROSCOPIC PERMEABILITY        *
*             COEFFICIENTS (PROGRAM MCPERM).                           *
*                                                                      *
*  PROGRAMMING NOTES  - DETAILS AS TO THE VARIABLE DEFINITIONS AND     *
*                       DESCRIPTION OF THE ALGORITHM ARE GIVEN         *
*                       THROUGHOUT THE PROGRAM TO FACILITATE ITS       *
*                       UNDERSTANDING.                                 *
*                                                                      *
*  VARIABLES                                                           *
*                                                                      *
*       NCASES - MAXIMUM NUMBER OF CASES THAT CAN BE PROCESSED BY      *
*                THE PROGRAM.                                          *
*                                                                      *
*        NREPS - MAXIMUM NUMBER OF REPLICATES THAT CAN BE PROCESSED    *
*                BY THE PROGRAM.                                       *
*                                                                      *
*        CALIB - REFERENCE VALUE CORRESPONDING TO THE READING          *
*                OBTAINED FROM THE PARTICLE COUNTER WITH THE           *
*                CALIBRATION BEADS (DIMENSIONLESS).                    *
*                                                                      *
*        GAINF - CORRECTION FACTOR FOR THE GAIN SETTING USED IN THE    *
*                MEASUREMENTS (DIMENSIONLESS).                         *
*                                                                      *
*       BEADVL - VOLUME OF THE BEADS USED FOR CALIBRATION, IN CM3.     *
*                                                                      *
*        TSTEP - TIME STEP BETWEEN EACH READING (ASSUMED CONSTANT),    *
*                IN SEC                                                *
*                                                                      *
*       TOTREP - TOTAL NUMBER OF REPLICATES TREATED BY THE PROGRAM.    *
*                                                                      *
*         NPTS - NUMBER OF POINTS TO BE CONSIDERED IN THE LINEAR       *
*                REGRESSION PROCEDURE TO ESTIMATE THE INITIAL          *
*                CELL VOLUME AND THE INITIAL CELL VOLUME CHANGE.       *
*                                                                      *
*       TOTCAS - TOTAL NUMBER OF CASES TREATED BY THE PROGRAM.         *
*                                                                      *
*         OBSM - MATRIX OF EXPERIMENTAL DATA. THEY ARE THE             *
*                ARITHMETIC AVERAGES OF THE OUTPUT VALUES OBTAINED     *
*                BY MEASURING A CERTAIN NUMBER OF INDIVIDUAL CELLS.    *
*                                                                      *
*        NINDM - MATRIX OF NUMBER OF CELLS USED TO ESTIMATE EACH OF    *
*                THE AVERAGE VOLUME READINGS. THE LATTER VALUE IS      *
*                USED AS "WEIGHT" IN SOME CALCULATIONS, THAT IS,       *
*                IT IS USED AS A MEASURE OF THE QUALITY OF ITS         *
*                ASSOCIATED VOLUME MEASUREMENT (DIMENSIONLESS).        *
*                                                                      *
*       MAXIND - MAXIMUM NUMBER OF INDIVIDUALS USED TO ESTIMATE A      *
*                MEAN IN A GIVEN SET.                                  *
*                                                                      *
*       GRMEAN - GRAND MEAN OF A SET OF REPLICATE MEANS.               *
*                                                                      *
*        NMEAN - NUMBER OF REPLICATES FOR ESTIMATING A GIVEN GRAND     *
*                MEAN.                                                 *
*                                                                      *
*         VARM - MATRIX OF ESTIMATES OF THE VARIANCE OF THE MEANS.     *
*                                                                      *
*   MAXV, MINV - VECTORS OF MAXIMUM AND MINIMUM VALUES OF THE          *
*                VARIANCE ESTIMATES ENCOUNTERED IN THE REPLICATE       *
*                SETS.                                                 *
*                                                                      *
*          WTM - MATRIX OF "WEIGHTS" ASSOCIATED TO THE OBSERVED        *
*                MEANS. THESE WEIGHTS ARE DEFINED AS THE INVERSE       *
*                OF THE ESTIMATED VARIANCES OF THE MEANS.              *
*                                                                      *
*         VOLM - MATRIX OF EXPERIMENTAL DATA IN VOLUME UNITS,          *
*                IN CM3.                                               *
*                                                                      *
* SUMW, SUMWY,   - VARIOUS SUMS USED IN THE CALCULATIONS.              *
* SUMWX, SUMWXY,                                                       *
* SUMWX2, SUM                                                          *
*                                                                      *
*       HAFSTP - HALF TIME STEP, IN SEC.                               *
*                                                                      *
*            X - CURRENT VALUE OF INDEPENDENT VARIABLE (TIME),         *
*                IN SEC.                                               *
*                                                                      *
*   YBAR, XBAR - WEIGHTED MEANS OF THE OBSERVATIONS AND OF THE         *
*                TIME VALUES, IN CM3 AND SEC RESPECTIVELY.             *
*                                                                      *
* INTERV, SLOPEV - VECTORS OF ESTIMATES OF THE CELL VOLUME AND OF      *
*                  THE CELL VOLUME CHANGE AT TIME 0 (IN CM3 AND        *
*                  CM3 SEC-1 RESPECTIVELY) FOR ALL REPLICATES.         *
*                                                                      *
*        NDATA - NUMBER OF CASES IN A GIVEN REPLICATE.                 *
*                                                                      *
************************************************************************

*     DEBUG UNIT(2), INIT
*     END DEBUG

      PROGRAM DATATR

      INTEGER NCASES, NREPS
      PARAMETER(NCASES = 200, NREPS = 8)

*     ROUTINE VARIABLES.

      REAL*8 CALIB, GAINF, BEADVL, TSTEP, OBSM(0:NCASES, NREPS),
     &       NINDM(0:NCASES, NREPS), MAXIND, GRMEAN, SUMW, SUMWY,
     &       SUM, VARM(0:NCASES, NREPS), MAXV(NREPS), MINV(NREPS),
     &       WTM(0:NCASES, NREPS), VOLM(0:NCASES, NREPS), SUMWX,
     &       SUMWXY, SUMWX2, HAFSTP, X, YBAR, XBAR, INTERV(NREPS),
     &       SLOPEV(NREPS)

      INTEGER TOTREP, NPTS, TOTCAS, NMEAN, NDATA

*     INPUT DATA.

      READ(1, *) CALIB, GAINF, BEADVL
      READ(1, *) TSTEP, TOTREP, NPTS

*     THE OBSERVED MEANS AND THE NUMBER OF INDIVIDUALS IN EACH MEAN
*     MUST BE IN THE FORM OF A NCASES BY (2*NREPS) MATRIX. FOR EACH
*     REPLICATE THE FIRST COLUMN CONTAINS THE OBSERVED MEANS AND THE
*     SECOND COLUMN, THE NUMBER OF INDIVIDUALS IN EACH MEAN.

      TOTCAS = 0
      DO 10 K = 1, NCASES-1
         READ(1, *, END=20) (OBSM(K,J), NINDM(K,J), J = 1, TOTREP)
         TOTCAS = TOTCAS + 1
10    CONTINUE
20    CONTINUE

*     ESTIMATE THE VARIANCES OF THE OBSERVED MEANS. THE INVERSE OF
*     THESE ESTIMATES WILL BE USED AS "WEIGHTS" IN THE REGRESSION
*     ANALYSES.

*     WHEN ONLY ONE SET IS AVAILABLE, THE WEIGHT FOR EACH MEAN OF
*     THAT SET IS TAKEN AS THE NUMBER OF CELLS USED TO ESTIMATE THAT
*     VALUE DIVIDED BY THE MAXIMUM NUMBER OF INDIVIDUALS ENCOUNTERED
*     IN THE SET.

      IF (TOTREP .EQ. 1) THEN

        MAXIND = 0.0D0
        DO 32 I = 1, TOTCAS
           MAXIND = MAX(MAXIND, NINDM(I,1))
32      CONTINUE

        WTM(0,1) = 1.0D0
        DO 34 I = 1, TOTCAS
           WTM(I,1) = NINDM(I,1) / MAXIND
34      CONTINUE

       ELSE

*       WHEN THERE IS MORE THAN ONE REPLICATE BUT, FOR SOME CASES,
*       ONLY ONE MEAN IS AVAILABLE, THE ESTIMATED VARIANCE FOR THAT
*       MEAN IS SET TO THE MAXIMUM VALUE ENCOUNTERED IN THE SET IT
*       BELONGS TO. ON THE OTHER HAND, FOR CASES WHERE THE ESTIMATED
*       VARIANCE IS ZERO, THE ESTIMATE IS SET TO THE MINIMUM VALUE
*       OF THE SET IT BELONGS TO.

        DO 30 I = 1, TOTCAS

           GRMEAN = 0.0D0
           SUMW = 0.0D0
           SUMWY = 0.0D0
           NMEAN = TOTREP

           DO 40 J = 1, TOTREP
              IF (OBSM(I,J) .EQ. 0.0D0) NMEAN = NMEAN - 1
              SUMW = SUMW + NINDM(I,J)
              SUMWY = SUMWY + (OBSM(I,J) * NINDM(I,J))
40         CONTINUE

           GRMEAN = SUMWY / SUMW

           SUM = 0.0D0
           DO 50 J = 1, TOTREP
              SUM = SUM + (NINDM(I,J) * ((OBSM(I,J) - GRMEAN)**2))
50         CONTINUE

           DO 60 J = 1, TOTREP
              IF (OBSM(I,J) .EQ. 0.0D0) GO TO 60
              IF (NMEAN .EQ. 1) THEN
                VARM(I,J) = -1.0D0
               ELSE
                VARM(I,J) = SUM / (REAL(NMEAN - 1) * NINDM(I,J))
              END IF
              IF (VARM(I,J) .EQ. 0.0D0) VARM(I,J) = -2.0D0
60         CONTINUE

30      CONTINUE

*       FIND, IN EACH REPLICATE SET, THE MAXIMUM AND MINIMUM
*       ESTIMATED VARIANCE.

        DO 70 J = 1, TOTREP
           MAXV(J) = -1.0D15
           MINV(J) = 1.0D15
           DO 70 I = 1, TOTCAS
              IF (VARM(I,J) .LT. 0.0D0) GO TO 70
              MAXV(J) = MAX(MAXV(J), VARM(I,J))
              MINV(J) = MIN(MINV(J), VARM(I,J))
70      CONTINUE

*       REPLACE THE CODED VARIANCES BY THE ABOVE EXTREMA (FOR ALL
*       REPLICATES) AND DEFINE THE "WEIGHTS".

        DO 80 J = 1, TOTREP
           VARM(0,J) = 1.0D0
           DO 80 I = 0, TOTCAS
              IF (OBSM(I,J) .EQ. 0.0D0) GO TO 80
              IF (VARM(I,J) .EQ. -1.0D0) VARM(I,J) = MAXV(J)
              IF (VARM(I,J) .EQ. -2.0D0) VARM(I,J) = MINV(J)
              WTM(I,J) = 1.0D0 / VARM(I,J)
80      CONTINUE

      END IF

*     TRANSFORM READINGS TO VOLUME UNITS.

      DO 90 J = 1, TOTREP
      DO 90 I = 1, TOTCAS
         VOLM(I,J) = ((GAINF * OBSM(I,J)) + BEADVL) / CALIB
90    CONTINUE

*     USING WEIGHTED LINEAR REGRESSION ON NPTS FIRST POINTS,
*     ESTIMATE INITIAL CELL VOLUME AND INITIAL CELL VOLUME CHANGE
*     FOR EACH REPLICATE.
```

```
       DO 100 J = 1, TOTREP

          SUMW = 0.0D0
          SUMWY = 0.0D0
          SUMWX = 0.0D0
          SUMWXY = 0.0D0
          SUMWX2 = 0.0D0

          DO 110 I = 1, NPTS
             SUMW = SUMW + WTM(I,J)
             SUMWY = SUMWY + (VOLM(I,J) * WTM(I,J))
             IF (I .EQ. 1) THEN
               HAFSTP = 0.5D0 * TSTEP
               X = HAFSTP
              ELSE
               X = X + TSTEP
             END IF
             SUMWX = SUMWX + (X * WTM(I,J))
             SUMWXY = SUMWXY + (VOLM(I,J) * X * WTM(I,J))
             SUMWX2 = SUMWX2 + ((X**2) * WTM(I,J))
110       CONTINUE

          YBAR = SUMWY / SUMW
          XBAR = SUMWX / SUMW

*         INITIAL CELL VOLUME CHANGE.

          SLOPEV(J)= (SUMWXY - ((SUMWX * SUMWY) / SUMW)) /
     &               (SUMWX2 - ((SUMWX**2) / SUMW))

*         INITIAL CELL VOLUME.

          INTERV(J) = YBAR - (SLOPEV(J) * XBAR)
          VOLM(0,J) = INTERV(J)

100    CONTINUE

*      OUTPUT RESULTS.

       WRITE(2, 2000) CALIB, BEADVL
2000   FORMAT(' CALIBRATION READING OF: ', F5.1, '  CORRESPONDS',
     &        ' TO A VOLUME OF: ', 1P, D10.3, ' CM3.')
       WRITE(2, 2010) GAINF
2010   FORMAT(/, ' CORRECTION FACTOR FOR GAIN SETTING: ', F5.3)
       DO 120 J = 1, TOTREP
          WRITE(2, 2020) J, INTERV(J), SLOPEV(J), TSTEP
2020      FORMAT(//, ' REPLICATE INDEX: ', I1, /,
     &            ' INITIAL CELL VOLUME: ', 1P, D10.3, ' CM3.', /,
     &            ' INITIAL CELL VOLUME CHANGE: ', D10.3,
     &            ' CM3 SEC-1.', /, ' TIME STEP: ', 0P, F5.1, //)
          WRITE(2, 2030)
2030      FORMAT(' CELL VOLUME  (CM3)          WEIGHT     TIME', /)
          NDATA = 0
          DO 130 I = 0, TOTCAS
             IF (VOLM(I,J) .EQ. 0.0D0) GO TO 140
             IF (I .EQ. 0) THEN
               X = 0.0D0
              ELSE IF (I .EQ. 1) THEN
                    X = X + HAFSTP
                   ELSE
                    X = X + TSTEP
             END IF
             WRITE(2, 2040) VOLM(I,J), WTM(I,J), X
2040         FORMAT(5X, 1P, D10.3, 10X, D10.3, 2X, 0P, F5.1)
             NDATA = NDATA + 1
130       CONTINUE
140       CONTINUE
          WRITE(2, 2050) NDATA
2050      FORMAT(/, ' TOTAL NUMBER OF [illegible]S: ', I4)
120    CONTINUE

       STOP
       END
```

```
*********************************************************************
*                                                                   *
*  PROGRAM NAME  - WMEAN                                            *
*                                                                   *
*  LATEST VERSION  - FEBRUARY 13, 1985.                             *
*                                                                   *
*  WRITTEN BY  - CHRISTIAN TOUPIN                                   *
*                FOOD ENGINEERING GROUP                             *
*                DEPARTMENT OF FOOD SCIENCE                         *
*                UNIVERSITY OF ALBERTA                              *
*                EDMONTON, ALBERTA.                                 *
*                                                                   *
*  PURPOSE  - THIS PROGRAM IS USED TO ESTIMATE THE GRAND MEAN OF    *
*             A SET OF SEVERAL MEANS WHICH, ON THEIR SIDE, ARE      *
*             ESTIMATED FROM SAMPLES CONTAINING DIFFERENT NUMBER    *
*             OF UNKNOWN INDIVIDUALS. IT ALSO ESTIMATES THE         *
*             STANDARD DEVIATION OF THAT GRAND MEAN.                *
*                                                                   *
*  PROGRAMMING NOTES  - DETAILS AS TO THE VARIABLE DEFINITIONS AND  *
*                       DESCRIPTION OF THE ALGORITHM ARE GIVEN      *
*                       THROUGHOUT THE PROGRAM TO FACILITATE ITS    *
*                       UNDERSTANDING.                              *
*                                                                   *
*  VARIABLES                                                        *
*                                                                   *
*     NCASES - MAXIMUM NUMBER OF CASES THAT CAN BE PROCESSED BY     *
*              THE PROGRAM.                                         *
*                                                                   *
*     TOTCAS - TOTAL NUMBER OF CASES TREATED BY THE PROGRAM.        *
*                                                                   *
* OBSV, NINDV - VECTOR OF OBSERVED MEANS AND VECTOR OF THE NUMBER   *
*              OF SAMPLE INDIVIDUALS IN EACH MEAN. THE LATTER       *
*              VALUES ARE USED AS "WEIGHTS" IN THE ESTIMATION OF    *
*              THE GRAND MEAN.                                      *
*                                                                   *
* SUMW, SUMWY, - VARIOUS SUMS USED IN THE CALCULATION OF THE        *
* SUM             WEIGHTED GRAND MEAN AND THE STANDARD DEVIATION OF *
*                 THE GRAND MEAN.                                   *
*                                                                   *
*       YBARH - WEIGHTED GRAND MEAN OF THE SET OF OBSERVED MEANS    *
*                                                                   *
*       STDEV - STANDARD DEVIATION OF THAT ESTIMATOR.               *
*                                                                   *
*********************************************************************

*     DEBUG UNIT(2), INIT
*     END DEBUG

      PROGRAM WMEAN

      INTEGER NCASES
      PARAMETER(NCASES = 1000)

*     ROUTINE VARIABLES

      REAL*8 OBSV(NCASES), NINDV(NCASES), SUMW, SUMWY, SUM, YBARH,
     &       STDEV

      INTEGER TOTCAS

*     INPUT DATA.

*     THE OBSERVED MEANS AND THE "WEIGHTS" MUST BE IN THE FORM OF
*     A NCASES BY 2 MATRIX (FIRST COLUMN: OBSERVED MEANS, SECOND
*     COLUMN: "WEIGHTS").

      TOTCAS = 0
      DO 10 K = 1, NCASES
         READ(1, *, END=20) OBSV(K), NINDV(K)
         TOTCAS = TOTCAS + 1
10    CONTINUE
20    CONTINUE

*     CALCULATE WEIGHTED GRAND MEAN AND STANDARD DEVIATION OF THAT
*     ESTIMATOR.

      SUMW = 0.0
      SUMWY = 0.0
      DO 30 K = 1, TOTCAS
         SUMW = SUMW + NINDV(K)
         SUMWY = SUMWY + (OBSV(K) * NINDV(K))
30    CONTINUE

*     WEIGHTED GRAND MEAN.

      YBARH = SUMWY / SUMW

      SUM = 0.0
      DO 40 K = 1, TOTCAS
         SUM = SUM + (NINDV(K) * ((OBSV(K) - YBARH)**2))
40    CONTINUE

*     STANDARD DEVIATION OF THAT ESTIMATOR.

      STDEV = SQRT(SUM / (REAL(TOTCAS - 1) * SUMW))

*     OUTPUT RESULTS.

      WRITE(2, *) ' OBSERVED MEAN  NUMBER OF SAMPLE'
      WRITE(2, *) '                  INDIVIDUALS'
      WRITE(2, *)
      DO 50 K = 1, TOTCAS
         WRITE(2, 2000) OBSV(K), NINDV(K)
2000  FORMAT(5X, F8.1, 11X, F8.1)
50    CONTINUE
      WRITE(2, 2010) TOTCAS, SUMW, YBARH, STDEV
2010  FORMAT(//, ' TOTAL NUMBER OF MEANS IN THE SET: ', I4, /,
     &       ' TOTAL NUMBER OF INDIVIDUALS: ', 1P, D10.3, //,
     &       ' WEIGHTED GRAND MEAN IS: ', 0P, F8.2, /,
     &       ' STANDARD DEVIATION OF THAT ESTIMATOR IS: ', 1P,D10.3)
      WRITE(2, 2020) SUMW * (STDEV**2), STDEV**2
2020  FORMAT(' ESTIMATED VARIANCE OF THE POPULATION OF INDIVIDUALS:'
     &    ,1X,1P,D10.3, /,' ESTIMATED VARIANCE OF THE GRAND MEAN: ',
     &      D10.3)

      STOP
      END
```

14.2 Input and output files

14.2.1 Program DATATR

Input file

The data necessary for program DATATR must observe the following format (the reader is referred to the program code for the definition of the variables). Note that *FREE FORMAT* input is used. This example corresponds to the average readouts of 3 runs performed on human granulocytes exposed to a 3x PBS solution at 10°C (see section 5.2.1).

```
113.5  0.511  4.828E-10                            CALIB  GAINF  DEADVL
1.0  3  5                                          TSTEP  TOTREP  NPTS
170.0   354.0   180.0   305.0   154.0   147.0      OBSM(K,J), NINDM(K,J)
161.0  2783.0   162.0  2712.0   161.0  3211.0          J = 1, TOTREP
154.0  2505.0   154.0  2956.0   153.0  2188.0          K = 1, TOTCAS
148.0  2483.0   147.0  2136.0   147.0  2121.0
145.0  2484.0   142.0  2333.0   143.0  2361.0
135.0  2440.0   136.0  2352.0   137.0  2369.0
133.0  2317.0   132.0  2388.0   132.0  2263.0
130.0  2416.0   128.0  2380.0   128.0  2405.0
123.0  2420.0   124.0  2376.0   124.0  2334.0
121.0  2378.0   121.0  2469.0   121.0  2366.0
118.0  2381.0   117.0  2398.0   117.0  2443.0
115.0  2399.0   114.0  2350.0   114.0  2401.0
114.0  2359.0   112.0  2355.0   112.0  2341.0
111.0  2388.0   110.0  2372.0   110.0  2438.0
108.0  2455.0   106.0  2421.0   108.0  2389.0
107.0  2413.0   106.0  2423.0   107.0  2367.0
105.0  2405.0   105.0  2374.0   105.0  2390.0
105.0  2366.0   105.0  2398.0   104.0  2381.0
104.0  2391.0   105.0  2435.0   103.0  2320.0
102.0  2411.0   103.0  2432.0   103.0  2328.0
103.0  2334.0   103.0  2484.0   102.0  2323.0
102.0  2300.0   102.0  2398.0   102.0  2407.0
101.0  2393.0   101.0  2394.0   101.0  2350.0
101.0  2355.0   102.0  2361.0   100.0  2388.0
100.0  2341.0   101.0  2374.0   100.0  2325.0
102.0  2469.0   100.0  2410.0   100.0  2304.0
 98.0  2407.0   100.0  2400.0    99.0  2463.0
100.0  2366.0    99.0  2415.0   100.0  2406.0
 98.0  2400.0    99.0  2431.0    98.0  2442.0
100.0  2405.0   101.0  2464.0    98.0  2453.0
 98.0  2393.0    99.0  2414.0    99.0  2401.0
 98.0  2373.0    99.0  2347.0    99.0  2383.0
 99.0  2392.0    99.0  2333.0    99.0  2385.0
 98.0  2425.0    98.0  2415.0    99.0  2372.0
 98.0  2445.0    98.0  2359.0    98.0  2341.0
 98.0  2366.0    98.0  2411.0    98.0  2360.0
 98.0  2377.0    98.0  2470.0    98.0  2421.0
 97.0  2444.0    98.0  2320.0    98.0  2413.0
 99.0  2382.0    98.0  2420.0    97.0  2366.0
 94.0  2382.0    98.0  2366.0    97.0  2405.0
 97.0  2452.0    98.0  2404.0    98.0  2376.0
 97.0  2447.0    98.0  2158.0    97.0  2425.0
 98.0  2370.0    97.0  2364.0    97.0  2404.0
 97.0  2403.0    98.0  2373.0    97.0  2406.0
 98.0  2424.0    97.0  2354.0    97.0  2356.0
 97.0  2330.0    97.0  2428.0    96.0  2370.0
 98.0  2343.0    98.0  2432.0    96.0  2233.0
 98.0  2392.0    97.0  2356.0    97.0  2303.0
 97.0  2433.0    98.0  2263.0    97.0  2327.0
 96.0  2341.0    98.0  2258.0    97.0  2408.0
 97.0  2411.0    98.0  2164.0    97.0  2409.0
 97.0  2466.0    97.0  2266.0    96.0  2456.0
 97.0  2244.0    95.0  2156.0    97.0  2325.0
 96.0  2208.0    97.0  2163.0    95.0  2323.0
 97.0  2185.0    96.0  2244.0    96.0  2181.0
 98.0  2215.0    96.0  2181.0    96.0  2168.0
 96.0  2116.0    97.0  2157.0    96.0  2158.0
 96.0  2180.0    96.0  2237.0    96.0  2164.0
 98.0  2215.0    96.0  2160.0    98.0  2154.0
 96.0  2138.0    98.0  2164.0    96.0  2134.0
```

Output file

The output resulting from the treatment of the previous data is presented here in a self-explanatory form. As can be seen, the average cellular volumes, their corresponding weights and the time of the measurements are in a format which is directly compatible with the input requirements of the program MCPERM.

```
CALIBRATION READING OF: 113.8  CORRESPONDS TO A VOLUME OF:  4.928E-10 CM3.

CORRECTION FACTOR FOR GAIN SETTING: 0.511

REPLICATE INDEX: 1
INITIAL CELL VOLUME:  3.784E-10 CM3.
INITIAL CELL VOLUME CHANGE: -1.262E-11 CM3 SEC-1.
TIME STEP.   1.0

CELL VOLUME  (CM3)          WEIGHT      TIME

     3.784E-10            1.000E+00     0.0
     3.768E-10            2.218E-02     0.5
     3.559E-10            2.953E+00     1.5
     3.414E-10            3.207E+00     2.5
     3.303E-10            7.893E-01     3.5
     3.214E-10            3.393E-01     4.5
     3.015E-10            3.061E+00     5.5
     2.948E-10            3.001E+00     6.5
     2.882E-10            7.527E-01     7.5
     2.727E-10            3.028E+00     8.5
     2.682E-10            3.272E+00     9.5
     2.638E-10            7.459E-01    10.5
     2.549E-10            3.010E+00    11.5
     2.527E-10            7.522E-01    12.5
     2.461E-10            2.993E+00    13.5
     2.416E-10            3.045E+00    14.5
     2.372E-10            2.997E+00    15.5
     2.350E-10            3.010E+00    16.5
     2.328E-10            3.002E+00    17.5
     2.305E-10            1.006E+00    18.5
     2.261E-10            3.013E+00    19.5
     2.283E-10            2.974E+00    20.5
     2.261E-10            3.272E+00    21.5
     2.239E-10            3.272E+00    22.5
     2.239E-10            9.860E-01    23.5
     2.217E-10            2.976E+00    24.5
     2.261E-10            7.818E-01    25.5
     2.172E-10            1.001E+00    26.5
     2.217E-10            2.951E+00    27.5
     2.172E-10            2.966E+00    28.5
     2.217E-10            4.198E-01    29.5
     2.172E-10            2.994E+00    30.5
     2.172E-10            3.008E+00    31.5
     2.195E-10            3.272E+00    32.5
     2.172E-10            3.047E+00    33.5
     2.172E-10            3.272E+00    34.5
     2.172E-10            3.272E+00    35.5
     2.172E-10            3.272E+00    36.5
     2.150E-10            3.033E+00    37.5
     2.195E-10            1.003E+00    38.5
     2.172E-10            2.964E+00    39.5
     2.150E-10            1.025E+00    40.5
     2.150E-10            3.272E+00    41.5
     2.128E-10            2.994E+00    42.5
     2.150E-10            3.025E+00    43.5
     2.128E-10            3.029E+00    44.5
     2.150E-10            2.945E+00    45.5
     2.128E-10            7.377E-01    46.5
     2.128E-10            3.027E+00    47.5
     2.150E-10            3.154E+00    48.5
     2.128E-10            2.982E+00    49.5
     2.150E-10            3.229E+00    50.5
     2.150E-10            3.051E+00    51.5
     2.150E-10            7.831E-01    52.5
     2.128E-10            9.807E-01    53.5
     2.150E-10            2.968E+00    54.5
     2.128E-10            3.272E+00    55.5
     2.106E-10            9.805E-01    56.5
     2.128E-10            3.272E+00    57.5
     2.128E-10            7.573E-01    58.5
     2.128E-10            3.272E+00    59.5

TOTAL NUMBER OF CASES:    61

REPLICATE INDEX: 2
INITIAL CELL VOLUME:  3.618E-10 CM3.
INITIAL CELL VOLUME CHANGE: -1.581E-11 CM3 SEC-1
TIME STEP:   1.0

CELL VOLUME  (CM3)          WEIGHT      TIME

     3.618E-10            1.000E+00     0.0
     3.547E-10            1.511E-02     0.5
     3.591E-10            2.908E+00     1.5
     3.414E-10            3.785E+00     2.5
     3.259E-10            8.845E-01     3.5
     3.148E-10            3.187E-01     4.5
     3.015E-10            2.951E+00     5.5
     2.926E-10            3.067E+00     6.5
     2.837E-10            7.408E-01     7.5
     2.749E-10            2.973E+00     8.5
     2.682E-10            3.785E+00     9.5
     2.594E-10            7.512E-01    10.5
     2.527E-10            2.948E+00    11.5
     2.483E-10            7.478E-01    12.5
     2.438E-10            2.973E+00    13.5
     2.394E-10            3.004E+00    14.5
     2.394E-10            3.010E+00    15.5
     2.328E-10            2.970E+00    16.5
     2.328E-10            3.017E+00    17.5
     2.328E-10            1.025E+00    18.5
     2.283E-10            3.039E+00    19.5
     2.283E-10            3.170E+00    20.5
     2.261E-10            3.785E+00    21.5
     2.239E-10            3.785E+00    22.5
     2.261E-10            9.885E-01    23.5
     2.239E-10            3.018E+00    24.5
     2.217E-10            7.437E-01    25.5
     2.217E-10            9.885E-01    26.5
     2.195E-10            3.012E+00    27.5
     2.195E-10            3.004E+00    28.5
     2.239E-10            4.300E-01    29.5
     2.195E-10            3.020E+00    30.5
     2.195E-10            2.975E+00    31.5
```

```
      2.195E-10            3.785E+00   32.5
      2.172E-10            3.034E+00   33.5
      2.172E-10            3.785E+00   34.5
      2.172E-10            3.785E+00   35.5
      2.172E-10            3.785E+00   36.5
      2.172E-10            2.879E+00   37.5
      2.172E-10            1.019E+00   38.5
      2.172E-10            2.986E+00   39.5
      2.172E-10            1.006E+00   40.5
      2.172E-10            2.888E+00   41.5
      2.150E-10            2.987E+00   42.5
      2.172E-10            2.867E+00   43.5
      2.150E-10            2.942E+00   44.5
      2.150E-10            3.071E+00   45.5
      2.172E-10            7.857E-01   46.5
      2.150E-10            2.981E+00   47.5
      2.172E-10            2.959E+00   48.5
      2.128E-10            2.857E+00   49.5
      2.172E-10            2.898E+00   50.5
      2.150E-10            2.804E+00   51.5
      2.106E-10            7.373E-01   52.5
      2.150E-10            8.895E-01   53.5
      2.128E-10            3.066E+00   54.5
      2.128E-10            3.785E+00   55.5
      2.150E-10            1.010E+00   56.5
      2.128E-10            3.785E+00   57.5
      2.128E-10            7.482E-01   58.5
      2.128E-10            3.785E+00   59.5

TOTAL NUMBER OF CASES:   61


REPLICATE INDEX: 3
INITIAL CELL VOLUME:  3.786E-10 CM3.
INITIAL CELL VOLUME CHANGE: -1.511E-11 CM3 SEC-1.
TIME STEP:    1.0


CELL VOLUME  (CM3)          WEIGHT     TIME

      3.786E-10            1.000E+00    0.0
      3.414E-10            8.209E-03    0.5
      3.559E-10            3.443E+00    1.5
      3.382E-10            2.801E+00    2.5
      3.259E-10            5.787E-01    3.5
      3.148E-10            3.253E-01    4.5
      3.037E-10            2.887E+00    5.5
      2.926E-10            2.931E+00    6.5
      2.837E-10            7.466E-01    7.5
      2.749E-10            2.920E+00    8.5
      2.662E-10            3.443E+00    9.5
      2.594E-10            7.653E-01   10.5
      2.527E-10            3.012E+00   11.5
      2.463E-10            7.433E-01   12.5
      2.438E-10            3.056E+00   13.5
      2.416E-10            2.839E+00   14.5
      2.372E-10            2.965E+00   15.5
      2.328E-10            2.990E+00   16.5
      2.305E-10            2.995E+00   17.5
      2.283E-10            8.762E-01   18.5
      2.283E-10            2.809E+00   19.5
      2.261E-10            2.984E+00   20.5
      2.261E-10            3.443E+00   21.5
      2.239E-10            3.443E+00   22.5
      2.217E-10            1.001E+00   23.5
      2.217E-10            2.855E+00   24.5
      2.217E-10            7.110E-01   25.5
      2.195E-10            1.021E+00   26.5
      2.217E-10            3.003E+00   27.5
      2.172E-10            3.018E+00   28.5
      2.172E-10            4.280E-01   29.5
      2.195E-10            3.004E+00   30.5
      2.195E-10            2.895E+00   31.5
      2.195E-10            3.443E+00   32.5
      2.195E-10            2.960E+00   33.5
      2.172E-10            3.443E+00   34.5
      2.172E-10            3.443E+00   35.5
      2.172E-10            3.443E+00   36.5
      2.172E-10            2.994E+00   37.5
      2.150E-10            8.868E-01   38.5
      2.150E-10            3.013E+00   39.5
      2.128E-10            8.842E-01   40.5
      2.150E-10            3.243E+00   41.5
      2.150E-10            3.037E+00   42.5
      2.150E-10            3.026E+00   43.5
      2.150E-10            2.844E+00   44.5
      2.128E-10            2.986E+00   45.5
      2.128E-10            7.031E-01   46.5
      2.150E-10            2.914E+00   47.5
      2.150E-10            3.016E+00   48.5
      2.150E-10            3.046E+00   49.5
      2.150E-10            3.226E+00   50.5
      2.128E-10            3.038E+00   51.5
      2.150E-10            7.906E-01   52.5
      2.106E-10            1.032E+00   53.5
      2.128E-10            2.982E+00   54.5
      2.128E-10            3.443E+00   55.5
      2.128E-10            1.010E+00   56.5
      2.128E-10            3.443E+00   57.5
      2.172E-10            7.482E-01   58.5
      2.128E-10            3.443E+00   59.5

TOTAL NUMBER OF CASES:   61
```

14.2.2 Program WMEAN

Since this program is rather straightforward to use, we refer the reader to the listing (see section 14.1) for details on the input format and the output information.

14.3 Remarks

It should be emphasized at this level that the previous programs (DATATR and WMEAN) have been designed to process experimental measurements of a particular form. This does not however rule out other sorts of data as would be produced, for example, by different experimental techniques. It is important to realize that, as far as the program MCPERM is concerned, the treatments that the data have undergone are irrelevant, provided that the pertinent information for the optimization run is available. The potential experimenter therefore has complete freedom to adapt these programs to fit his or her needs.

15. APPENDIX 4: NUMERICAL SOLUTION OF THE MODEL EQUATIONS

This appendix describes the procedures and techniques required to solve *numerically* the system of equations presented in Chapter 8. We discuss firstly how further generalization can be achieved by performing a *dimensional analysis* on the model equations, then, we tackle the question of the solution of the system of equations by describing in detail the *numerical techniques* used to carry out the simulations.

15.1 Dimensional analysis

To facilitate the mathematical solution of the system of equations presented in Chapter 8, a *dimensional analysis* is carried out on the aforementioned relations. This procedure has the main advantage of *generalizing* the model by rendering the terms involved in the equations *independent* of any system of units, that is, it converts all variables in *dimensionless form* and possibly can create dimensionless groups that characterize the phenomenon under consideration.

Since the technique is relatively straightforward (it consists basically of performing simple algebraic rearrangements), we will restrict the discussion to presenting the dimensionless variables used to carry out the analysis and the final equations obtained through such a process. The reader is referred to Bird *et al.* (1960) and Loncin and Merson (1979) for more details on the general topic of dimensional analysis of equations of change.

15.1.1 Transmembrane exchanges

Referring to section 8.2.1, the definition of the thermodynamic forces involved in transmembrane and symplastic transport and the ensuing flux equations are transformed as follows[24].

Selecting for convenience the conditions at *full turgor* (subscript 0) as *characteristic* values for the variables related to the tissue properties, one defines (for the j-th ECUC):

1. The reduced intracellular, extracellular and bath concentrations of species i:

$$\underline{c}c_i = cc_i \;/\; c_i* \qquad [A4.1]$$

$$\underline{c}i_i = ci_i \;/\; c_i* \qquad [A4.2]$$

and

$$\underline{c}b_i = cb_i \;/\; c_i* \qquad [A4.3]$$

where c_i* is the *reference* concentration for species i, defined as:

$$c_i* = .5\;[cc_{io} + cb_i(\theta=0)] \qquad [A4.4]$$

2. The reduced thermodynamic forces for transmembrane and symplastic transport of species i:

---

[24] Let us remind the reader that *underscore*, "_", will be used to differentiate a variable in *reduced* (dimensionless) form from its unit-dependent equivalent. The *slash*, "/", on the other hand, will indicate a *modified* form that still carries some units.

$$\Delta\underline{\mu}m_i = \Delta\mu m_i \;/\; \mathcal{R}T \qquad [A4.5]$$

and

$$\Delta\underline{\mu}p_i^{-(+)} = \Delta\mu p_i^{-(+)} \;/\; \mathcal{R}T \qquad [A4.6]$$

where T is the absolute temperature of the system.

3. The *modified* macroscopic phenomenological permeabilities (Onsager form) of the plasmalemma and of the plasmodesma pseudo-membrane for the pair i-k:

$$\mathcal{L}m_{ik} = \varnothing m \, Lm_{ik} \text{ for } i=k \text{ and } i\neq k \text{ forms} \qquad [A4.7]$$

and

$$\mathcal{L}p_{ik}^{-(+)} = \varnothing p \, Lp_{ik}^{-(+)} \text{ for } i=k \text{ and } i\neq k \text{ forms} \qquad [A4.8]$$

where $\varnothing m$ and $\varnothing p$ are *normalization factors* defined respectively as:

$$\varnothing m = Am_0 \, \mathcal{R}T \;/\; Vc_0 \qquad [A4.9]$$

and

$$\varnothing p = Ap'_0 \, \mathcal{R}T \;/\; Vc_0 \qquad [A4.10]$$

where Ap'_0 is the fraction of total symplastic transfer area pertaining to the (j,j-1)-th and (j,j+1)-th interface at full turgor (see equations 8.83 and 8.85). Note that both areas are equal since at full turgor all cells are identical.

4. Finally, the *modified* transmembrane and symplastic fluxes of species i:

$$\not{J}m_i = (\phi m/\mathcal{R}T)\ Jm_i \qquad [A4.11]$$

and

$$\not{J}p_i^{-(+)} = (\phi p/\mathcal{R}T)\ Jp_i^{-(+)} \qquad [A4.12]$$

Applying the principles of dimensional analysis, equations 8.59 and 8.60 are then rewritten in terms of these reduced or modified variables, leading to, after proper rearrangements (for transmembrane fluxes):

$$\not{J}m_i = -\Sigma_k \not{L}m_{ki}\ \Delta\underline{\mu}m_k\ ;\ i,k = 1,2,\ldots,m \qquad [A4.13]$$

where, for the solvent (i = 1), from relation 8.49 (referring to equations 8.38, 8.39, 8.40, 8.43, 8.44 and 8.47):

$$\Delta\underline{\mu}m_1 \cong -\tilde{v}_1\ \Sigma_i C_i{*}\ (\underline{c}c_i - \underline{c}i_i) + \tilde{v}_1 \not{P}c$$
$$i = 2,3,\ldots,m \qquad [A4.14]$$

and, for all other species but water, i.e. for $i = 2,3,\ldots,m$:

$$\Delta\underline{\mu}m_i \cong (\underline{c}c_i - \underline{c}i_i)\ /\ \bar{\underline{c}}m_i + \tilde{v}_i \not{P}c \qquad [A4.15]$$

where $\bar{\underline{c}}m_i$ is the dimensionless form of the average concentration of species i across the plasmalemma, given by (see equation 8.40):

$$\bar{\underline{c}}m_i = .5\ (\underline{c}c_i + \underline{c}i_i) \qquad [A4.16]$$

The *modified* hydrostatic pressure, $\not{P}c$, is defined by performing similar alterations to equations 8.43, 8.44 and

8.47, leading to:

$$\not{P}c = \{\not{P}c_0 + [\beta_2/(\mathcal{R}T\ \beta_1)]\}\underline{V}c\uparrow\beta_1 - [\beta_2/(\mathcal{R}T\ \beta_1)] \qquad [A4.17]$$

when ξ is dependent on the internal hydrostatic pressure, or to:

$$\not{P}c = (\xi/\mathcal{R}T)\ \ln \underline{V}c + \not{P}c_0 \qquad [A4.18]$$

when ξ is assumed constant. The modified pressure at full turgor, $\not{P}c_0$, is given by (see equation 8.47):

$$\not{P}c_0 = \Sigma_k C_k{*}\ (\underline{C}c_{k\,o} - \underline{C}i_{k\,o}) \qquad [A4.19]$$

where the summation runs for all the impermeable solutes present in the intra- and extracellular solutions at full turgor. Note that we have introduced here the reduced cellular volume, which is defined as:

$$\underline{V}c = Vc\ /\ Vc_0 \qquad [A4.20]$$

Turning now to the symplastic fluxes, equation 8.60 leads similarly to:

$$\not{J}p_i^- = -\Sigma_k \text{Ł}p_{k\,i}^-\ \Delta\underline{\mu}p_k^- \qquad i,k = 1,2,\ldots,m \qquad [A4.21]$$

where, as for equations A4.14, A4.15 and A4.16, for the solvent water:

$$\Delta\underline{\mu}p_1^- \cong -\tilde{v}_1\ \Sigma_i C_i{*}\ (\underline{C}c_i^{\,j} - \underline{C}c_i^{\,j-1}) + \tilde{v}_1\Delta\not{P}p^- \qquad i = 2,3,\ldots,m \qquad [A4.22]$$

and, for the other components:

$$\Delta\underline{\mu}p_i^- = (\underline{c}c_i^{\,j} - \underline{c}i_i^{\,j-1}) \;/\; \bar{\underline{c}}p_i^- + \tilde{v}_i\Delta\not{P}p^-$$

$$i = 2,3,\ldots,m \qquad [A4.23]$$

where the reduced average concentration of species i across the plasmodesmata linking the j-th and (j-1)-th cell, $\bar{\underline{c}}p_i^-$, is given by:

$$\bar{\underline{c}}p_i^- = .5\;(\underline{c}c_i^{\,j} + \underline{c}c_i^{\,j-1}) \qquad [A4.24]$$

and the difference in hydrostatic pressure (modified form) is defined as:

$$\Delta\not{P}p^- = \not{P}c^{\,j} - \not{P}c^{\,j-1} \qquad [A4.25]$$

where $\not{P}c^{\,j}$ and $\not{P}c^{\,j-1}$ are evaluated using equations A4.17, A4.18 and A4.19. Note that, as for section 8.2.1, the definitions pertaining to the (j,j+1)-th cell interaction are obtained by replacing the supercript - by + in relations A4.21 to A4.25.

15.1.2 Interstitium equations of continuity

Let us define, again using the conditions at full turgor as reference values:

1. The reduced distance coordinate, $\underline{z}$, and its derivative:

$$\underline{z} = z \;/\; \ell_0 \text{ and } d\underline{z} = dz \;/\; \ell_0 \qquad [A4.26]$$

2. The reduced time, $\underline{\theta}$, and its derivative:

$$\underline{\theta} = \theta / \theta* \text{ and } d\underline{\theta} = d\theta / \theta* \qquad \text{[A4.27]}$$

where $\theta*$ is the *reference* time, defined as:

$$\theta* = \ell_0^2 / D\blacksquare^{m}_{\infty} \qquad \text{[A4.28]}$$

where $D\blacksquare^{m}_{\infty}$ is the *geometrical mean* of the binary diffusion coefficients at *infinite dilution* of the solutes present in the system, i.e.:

$$D\blacksquare^{m}_{\infty} = (\Pi_i D\blacksquare_{i\infty})^{1/(m-1)} \; ; \; i = 2,3,\ldots,m \qquad \text{[A4.29]}$$

3. The reduced volume average velocity, $\underline{v}\blacksquare$, and its derivative:

$$\underline{v}\blacksquare = v\blacksquare / v\blacksquare* \text{ and } d\underline{v}\blacksquare = dv\blacksquare / v\blacksquare* \qquad \text{[A4.30]}$$

where $v\blacksquare*$ is the *reference* velocity given by:

$$v\blacksquare* = D\blacksquare^{m}_{\infty} / \ell_0 \qquad \text{[A4.31]}$$

4. The reduced interstitium volume, $\underline{V}i$, and the reduced plasmalemma transfer area, $\underline{A}m$:

$$\underline{V}i = Vi / Vi_0 \text{ and } d\underline{V}i = dVi / Vi_0 \qquad \text{[A4.32]}$$

and

$$\underline{A}m = Am / Am_0 \qquad \text{[A4.33]}$$

Substituting these variables (along with the definition of the reduced interstitium concentration of species i [relation A4.2]) in equation 8.78 yields, on rearrangement:

$$\partial\underline{C}i_i/\partial\underline{\theta} =$$
$$\partial/\partial\underline{z}(\underline{\mathcal{D}}^{\blacksquare}_i\ \partial\underline{C}i_i/\partial\underline{z}) - \underline{v}^{\blacksquare}\ \partial\underline{C}i_i/\partial\underline{z} -$$
$$[\partial\underline{v}^{\blacksquare}/\partial\underline{z} + (\theta*/\underline{V}i)\partial\underline{V}i/\partial\theta]\underline{C}i_i - \underline{R}_i \qquad [A4.34]$$

where $\underline{\mathcal{D}}^{\blacksquare}_i$ is the reduced form of the *corrected* pseudo-binary diffusivity of species i, defined as:

$$\underline{\mathcal{D}}^{\blacksquare}_i = \mathcal{D}^{\blacksquare}_i \ / \ D^{\blacksquare m}_{\infty} \qquad [A4.35]$$

and $\underline{R}_i$ is the reduced *production-depletion term* for species i, given by:

$$\underline{R}_i = \{[\theta* \ (1-\epsilon'_0)/\epsilon'_0](\underline{A}m/\underline{V}i)(\not{J}m_i/C_i*)\} \qquad [A4.36]$$

where the transmembrane flux appearing in the last term of equation 8.78 has been replaced by its modified form (equation A4.11), and the ratio $(1-\epsilon'_0)/\epsilon'_0$, where ϵ'_0 is the tissue void fraction at full turgor, has been substituted for the ratio Vc_0/Vi_0, which appears when one introduces equations A4.9 and A4.32 into the development (see equation 8.3). Note that the time variable, θ, appearing in the third term of equation A4.34 is *not* in dimensionless form. We will return to this point in the next section.

Similarly, equation 8.72 can be transformed to lead to:

$$\partial\underline{v}^{\blacksquare}/\partial\underline{z} = -[\underline{R}v + (\theta*/\underline{V}i)\partial\underline{V}i/\partial\theta] \qquad [A4.37]$$

where $\underline{R}v$ is the reduced *volumetric production-depletion term*, given by:

$$\underline{R}v = \{[\theta^* (1-\epsilon'_o)/\epsilon'_o](\underline{A}m/\underline{V}i)\Sigma_i \hat{v}_i \phi m_i\}$$

$$i = 1,2,\ldots,m \qquad [A4.38]$$

15.1.3 Intracellular equations of change

To carry out dimensional modifications on the intracellular equations of change, we need to introduce:

1. The reduced cellular volume, $\underline{V}c$, and its derivative, and the reduced cellular nonosmotic volume, $\underline{V}d$:

$$\underline{V}c = Vc / Vc_o \text{ and } d\underline{V}c = dVc / Vc_o \qquad [A4.39]$$

and

$$\underline{V}d = Vd / Vc_o \qquad [A4.40]$$

2. The reduced fractional symplastic transfer area:

$$\underline{A}p^{-(+)} = Ap^{-(+)} / Ap'_o \qquad [A4.41]$$

3. The reduced interstitium radius:

$$\underline{R}i = Ri / Ri_o \qquad [A4.42]$$

By making use of relations A4.26 and A4.1, along with definitions A4.9-A4.12 and 8.24, one obtains, from equation 8.90:

$$\begin{aligned} d\underline{c}c_i/d\theta = {} & [\underline{R}i/(\underline{V}c-\underline{V}d)] \int(\phi m_i/c_i^*)d\underline{z} - [\underline{c}c_i/(\underline{V}c-\underline{V}d)]\, d\underline{V}c/d\theta + \\ & [\underline{A}p^-/(\underline{V}c-\underline{V}d)]\, (\phi p_i^-/c_i^*) + \\ & [\underline{A}p^+/(\underline{V}c-\underline{V}d)]\, (\phi p_i^+/c_i^*) \end{aligned} \qquad [A4.43]$$

and from equation 8.89:

$$d\underline{V}c/d\theta =$$

$$\underline{R}i \int \Sigma_i \tilde{v}_i \mathcal{J}m_i \, d\underline{z} + \underline{A}p^- \, \Sigma_i \tilde{v}_i \mathcal{J}p_i^- + \underline{A}p^+ \, \Sigma_i \tilde{v}_i \mathcal{J}p_i^+$$

$$i = 1,2,\ldots,m \qquad [A4.44]$$

where the integration is carried out in both equations over the entire *reduced* length of the j-th cell, i.e. $\underline{\ell} = \ell / \ell_0$.

It should be emphasized here that the time variable, θ, appearing in the above equations has not been transformed to its reduced form, as defined by relation A4.27. The main reason for this intentional omission is that the reference time introduced in equation A4.28 has no "operational" meaning when applied to the intracellular equations of change, since diffusion is not allowed in the cellular solution as opposed to the interstitial volume. The use of the dimensionless time would therefore introduce terms in these modified equations that could not be eliminated by simplification, rendering them unnecessarily cumbersome. We propose therefore to deal with the cellular equations and, in fact, as we will see in the next section, with all the *time*-based *ordinary differential equations* of the system, on a *real time basis* and to correct them when the information is linked to the interstitium equations, as is done, for example, in the production-depletion terms appearing in equations A4.36 and A4.38, where $\theta*$ is introduced to make the expressions dimensionless. We will return to this question in sections 15.3 and 15.4.

15.1.4 Supplementary equations of change

Geometrical relations

The relations which describe the time variation of the mensurations of the equivalent cylindrical cell form a set of ordinary differential equations that needs to be solved simultaneously to keep track of the structural reorganization caused by the dehydration process. As discussed in section 15.1.3, this system will be dealt with on a *real time basis* since the time variable appearing in these relations is not transformed to its reduced form. The *modified* analogs of the geometrical equations of change are thus obtained as follows. Introducing:

1. The reduced ECUC length, $\underline{\ell}$, and its derivative:

$$\underline{\ell} = \ell / \ell_0 \text{ and } d\underline{\ell} = d\ell / \ell_0 \qquad \text{[A4.45]}$$

2. The reduced total ECUC volume, $\underline{V}$, and its derivative:

$$\underline{V} = V / V_0 \text{ and } d\underline{V} = dV / V_0 \qquad \text{[A4.46]}$$

3. The reduced cellular and buffer cylinder radii, $\underline{R}c$ and $\underline{R}b$, and their respective derivatives:

$$\underline{R}c = Rc / Rc_0 \text{ and } d\underline{R}c = dRc / Rc_0 \qquad \text{[A4.47]}$$

and

$$\underline{R}b = Rb / Rb_0 \text{ and } d\underline{R}b = dRb / Rb_0 \qquad \text{[A4.48]}$$

4. The reduced apoplastic transfer area, $\underline{A}i$, and its

derivative:

$$\underline{A}i = Ai \,/\, Ai_o \text{ and } d\underline{A}i = dAi \,/\, Ai_o \qquad [\text{A4.49}]$$

and, referring to reduced variables appearing in sections 15.1.1-3, equations 8.92, 8.93, 8.94, 8.95, 8.100 and 8.101 (see section 8.2.4), become, after being rewritten in terms of the above dimensionless variables, respectively:

$$d\underline{\ell}/d\theta = (1/3)\, \underline{V}^{-2/3}\, d\underline{V}/d\theta \qquad [\text{A4.50}]$$

for the time variation of the reduced ECUC length,

$$d\underline{R}i/d\theta = (\underline{\ell}^{-1}\, d\underline{A}m/d\theta - \underline{A}m\, \underline{\ell}^{-2}\, d\underline{\ell}/d\theta) \qquad [\text{A4.51}]$$

for the time variation of the reduced interstitium cylinder radius,

$$\begin{aligned} d\underline{R}c/d\theta &= \\ &(Ri_o/Rc_o)^2\, (\underline{R}i/\underline{R}c)\, d\underline{R}i/d\theta + [Vc_o/(2\pi\, Rc^2{}_o\, \ell_o)] \\ &[(\underline{R}c\, \underline{\ell})^{-1}\, d\underline{V}c/d\theta - \underline{V}c\, \underline{R}c^{-1}\, \underline{\ell}^{-2}\, d\underline{\ell}/d\theta] \qquad [\text{A4.52}] \end{aligned}$$

for the time variation of the reduced cellular cylinder radius,

$$\begin{aligned} d\underline{R}b/d\theta &= \\ &(Ri_o/Rb_o)^2\, (\underline{R}i/\underline{R}b)\, d\underline{R}i/d\theta - [Vi_o/(2\pi\, Rb^2{}_o\, \ell_o)] \\ &[(\underline{R}b\, \underline{\ell})^{-1}\, d\underline{V}i/d\theta - \underline{V}i\, \underline{R}b^{-1}\, \underline{\ell}^{-2}\, d\underline{\ell}/d\theta] \qquad [\text{A4.53}] \end{aligned}$$

for the time variation of the reduced buffer cylinder radius,

$$d\psi i/d\theta = (1 - \psi w)(\phi w'_o/\epsilon'_o)\, \underline{V}i^{-2}\, d\underline{V}i/d\theta \qquad [\text{A4.54}]$$

for the time variation of the interstitium diffusibility, where we have introduced the reduced cell wall volume, $\underline{V}w$, defined as:

$$\underline{V}w = Vw / Vw_o = Vw_o / Vw_o = 1 \qquad [A4.55]$$

since this volume remains constant (at its reference value) at all times, and replaced the ratio Vw_o/Vi_o, which emerges during the development by the ratio $\phi w'_o/\epsilon'_o$. Finally, one obtains for the time variation of the reduced apoplastic transfer area:

$$d\underline{A}i/d\theta = (2\pi/Ai_o)(Ri^2_o\, \underline{R}i\, d\underline{R}i/d\theta - Rb^2_o\, \underline{R}b\, d\underline{R}b/d\theta) \qquad [A4.56]$$

Changes during dehydration

The reduced interstitium and total volume changes of the ECUC are related to the reduced cellular volume variation as follows (referring to equations 8.102 and 8.103):

$$d\underline{V}i/d\theta = \kappa\, [(1-\epsilon'_o)/\epsilon'_o]\, d\underline{V}c/d\theta \qquad [A4.57]$$

and

$$d\underline{V}/d\theta = (1+\kappa)(1-\epsilon'_o)\, d\underline{V}c/d\theta \qquad [A4.58]$$

where we have replaced the ratio Vc_o/Vi_o by $(1-\epsilon'_o)/\epsilon'_o$, since, by definition (see equation 8.31), $\epsilon'_o = Vi_o/V_o$ and $V_o = Vi_o+Vc_o$. The time variations of the reduced plasmalemma and symplastic transfer areas are given for their part by (for stage 1):

$$d\underline{A}m/d\theta = (2/3)\ \underline{V}c^{-1/3}\ d\underline{V}c/d\theta \qquad [A4.59]$$

and

$$d\underline{A}p/d\theta = d\underline{A}m/d\theta \qquad [A4.60]$$

or, in terms of $d\underline{A}p'$, the change in the *fractional* symplastic transfer area[27]:

$$d\underline{A}p'/d\theta = d\underline{A}p/d\theta = d\underline{A}m/d\theta \qquad [A4.61]$$

and by:

$$d\underline{A}p/d\theta = d\underline{A}p'/d\theta = d\underline{A}m/d\theta = 0 \qquad [A4.62]$$

for stages 2 and 3.

Overall mass variation

Introducing the reduced total mass and the reduced mass of species i in the tissue, $\underline{m}'$ and $\underline{m}_i$, and their respective derivatives:

$$\underline{m}' = m' / m'_o \text{ and } d\underline{m}' = dm' / m'_o \qquad [A4.63]$$

and

$$\underline{m}_i = m_i / m'_o \text{ and } d\underline{m}_i = dm_i / m'_o \qquad [A4.64]$$

and defining the reduced apoplastic molar flux for the i-th species as:

---

[27] It is in fact more convenient to use relation A4.61 for solving the model equations since all symplastic transfer areas involved in the model are *fractions* of total areas (see equations A4.43, A4.44, A4.67, A4.68 and A4.71).

$$\underline{N}i_i = Ni_i / Ni_i* \qquad [A4.65]$$

where the reference flux, Ni_i* is given by:

$$Ni_i* = c_i* \, v_{\blacksquare}* \qquad [A4.66]$$

one obtains, on rearrangement of equations 8.112 and 8.107, for the variation of the reduced total mass of the tissue:

$$
\begin{aligned}
& d\underline{m}'/d\underline{\theta} = \\
& \quad -(\#col')(Vi_o/m'_o)(M_1/\tilde{v}_1)\ \underline{A}i|_{.}\ \{\underline{v}_{\blacksquare} - \\
& \quad \Sigma_i[\tilde{v}_i - (\tilde{v}_1 M_i/M_1)]c_i*\ \underline{N}i_i\}|_{.} + \\
& \quad (\#col')(Vc_o\ \theta*/m'_o)(\underline{A}p^-\ \Sigma_i M_i \not{J}p_i^-)|_{.} \\
& \quad i = 2,3,\ldots,m \qquad [A4.67]
\end{aligned}
$$

and for the net intake or loss of species i in the tissue:

$$
\begin{aligned}
& d\underline{m}_i/d\underline{\theta} = \\
& \quad -(\#col')(Vi_o/m'_o)\ \underline{A}i|_{.}\ M_i\ c_i*\ \underline{N}i_i|_{.} + \\
& \quad (\#col')(Vc_o\ \theta*/m'_o)(\underline{A}p^-\ M_i\ \not{J}p_i^-)|_{.} \qquad [A4.68]
\end{aligned}
$$

Note that the time variable is in *reduced* form in both equations.

15.1.5 Initial and boundary conditions

Initial conditions

Since we have selected both the *initial* and *reference* state of the tissue to correspond to the properties of the structure at *full turgor* (see sections 8.2.5 and 15.1.1), it follows that the conditions in the representative column at $\underline{\theta} = 0$ are, by definition:

1. For all #c cells, i.e. for j = 1,2,...,#c: $\underline{R}b^j = 1$; $\underline{R}i^j = 1$; $\underline{R}c^j = 1$; $\underline{\ell}^j = 1$; $\underline{A}m^j = 1$; $\underline{A}p^j = 1$ and accordingly $\underline{A}p^{j\prime} = 1$; $\underline{A}i^j = 1$; $\underline{V}c^j = 1$; $\underline{V}i^j = 1$; $\underline{V}^j = 1$ and $\psi i^j = \psi i_o$.

2. For all #c cells and all solutes present in the system, i.e. for j = 1,2,...,#c and i = 1,2,...,m: $\underline{c}c_i^j = \underline{c}c_{io}$ and $\underline{c}i_i^j = \underline{c}i_{io}$.

3. For all fluxes which can occur in the structure: $\mathcal{J}m_i = 0$, $\mathcal{J}p_i^{-(+)} = 0$, $\underline{N}i_i = 0$ and accordingly $\underline{v}^{\blacksquare} = 0$ for i = 1,2,...,m, and

4. For the initial mass of the tissue and of the species present in it: $\underline{m}^t = 1$ and $\underline{m}_i = \underline{m}_{io}$.

Boundary conditions

Referring to section 8.2.5, the boundary conditions are rewritten in dimensionless form as follows, for $\underline{\theta} > 0$:

1. For the conditions at the surface (i = 1,2,...,m):

$$\begin{aligned}&(\partial\underline{c}i_i/\partial\underline{z})|_s = \\ &\quad [(\underline{v}^{\blacksquare}/\underline{B}^{\blacksquare}_i)\underline{c}i_i]|_s - \\ &\quad [K_i/(v^{\blacksquare *}\,\underline{B}^{\blacksquare}_i|_s)](\underline{c}i_i|_s - \underline{c}b_i) \qquad [A4.69]\end{aligned}$$

if there is resistance to mass transfer at the surface or

$$\underline{c}i_i|_s = \underline{c}b_i \qquad [A4.70]$$

when $K_i \rightarrow \infty$. Note that, for $\underline{\theta} > 0$, $\underline{c}b_i = \underline{c}b_i(\underline{\theta}=0)$. For its part, the volume average velocity at the surface is given in reduced form by:

$$\underline{v}^{\blacksquare}|_{s} =$$
$$[\theta* (1-\epsilon'_{o})/\epsilon'_{o}][(\underline{A}p^{-}/\underline{A}i)\Sigma_{i}\tilde{v}_{i}\not\partial p_{i}^{-}]|_{s} -$$
$$(\theta*/\epsilon'_{o})(1/\underline{A}i)|_{s}\ \Sigma_{j}d\underline{V}^{j}/d\theta$$
$$i = 1,2,\ldots,m \text{ and } j = 1,2,\ldots,\#c \qquad [A4.71]$$

2. For the conditions at the center, we find for the semi-infinite body:

$$\underline{C}i_{i}|_{o} = \underline{C}i_{io} \text{ and } \underline{C}c_{i}|_{o} = \underline{C}c_{io}$$
$$i = 1,2,\ldots,m \qquad [A4.72]$$

and for the infinite slab:

$$(\partial\underline{C}i_{i}/\partial\underline{z})|_{o} = 0 \ ; \ i = 1,2,\ldots,m \qquad [A4.73]$$

along with the following conditions pertaining to both geometries:

$$\not\partial p_{i}^{+}|_{o} = 0 \ ; \ i = 1,2,\ldots,m \qquad [A4.74]$$

and

$$(\partial\underline{v}^{\blacksquare}/\partial\underline{z})|_{o} = 0 \text{ and } \underline{v}^{\blacksquare}|_{o} = 0 \qquad [A4.75]$$

3. For the conditions at the interface separating two adjoining cells, we have from equations 8.125 and 8.126:

$$(\underline{A}i^{j}\ \underline{v}^{\blacksquare j})|_{in} = (\underline{A}i^{j+1}\ \underline{v}^{\blacksquare j+1})|_{in} \qquad [A4.76]$$

and

$$(\underline{A}i^{j}\ \underline{B}^{\blacksquare}{}_{i}{}^{j}\ \partial\underline{C}i_{i}{}^{j}/\partial\underline{z})|_{in} =$$

$$(\underline{A}i^{j+1}\ \underline{B}^{\blacksquare}{}_{i}{}^{j+1}\ \partial\underline{C}i_{i}{}^{j+1}/\partial\underline{z})|_{in} \qquad [A4.77]$$

where we have assumed that for all species, i.e. for $i = 1,2,\ldots,m$:

$$\underline{C}i_{i}{}^{j}|_{in} = \underline{C}i_{i}{}^{j+1}|_{in} \qquad [A4.78]$$

15.2 Extracellular equations of change: the Crank-Nicolson scheme

The *partial differential equation* (*PDE*) describing the transport of species i in the interstitial volume of the j-th ECUC composing the hypothetical representative column (equation A4.34) is a highly *nonlinear parabolic PDE* since the terms $\underline{B}^{\blacksquare}{}_{i}$, $\underline{v}^{\blacksquare}$, $\partial\underline{v}^{\blacksquare}/\partial\underline{z}$, $\partial\underline{V}i/\partial\theta$ and $\underline{R}_{i}$ are *implicitly* functions of the interstitium concentration, $\underline{C}i_{i}$ (Carnahan, Luther and Wilkes, 1969). This relation, which cannot be solved *analytically*, must then be integrated using a *numerical technique* if one wishes to obtain the transient profile of concentration of the i-th species in the interstitium.

15.2.1 General finite-difference analogs

The analytical solution of equation A4.34 specifies $\underline{C}i_{i}$ as a function of 2 *independent continuous* variables, namely, the *distance* variable $\underline{z}$ and the *time* variable $\underline{\theta}$. To obtain a numerical solution, one replaces these continuous variables with *discrete finite-difference* equations, and it is these finite-difference equations which are solved numerically.

One must however obtain, as a first step, relations between finite-difference ratios and derivatives, to convert the differential equation of interest (written in terms of *continuous* variables) to a set of *finite-difference analogs* which will be used to carry out the integration.

When using a finite-difference technique to solve a PDE (plus its associated initial and boundary conditions), a network of *grid points* is established throughout the regions of interest occupied by the independent variables. For the problem under consideration, referring to Figure A4.1a, this region consists, for the distance variable $\underline{z}$, of the *extra-cellular continuum* formed by the linear arrangement of adjoining interstitial volumes pertaining to each ECUC comprising the column (see Figure 8.5) and, for the time variable $\underline{\theta}$, of a domain increasing without limit from zero. Thus, the portion of the $(\underline{z},\underline{\theta})$ plane described is a semi-infinite strip in the positive half-plane between the lines $\underline{z} = 0$ (center) and $\underline{z} = \Sigma_j \underline{\ell}^j$ for $j = 1,2,\ldots,\#c$ (surface).

When the two independent variables are replaced by discrete ones (also called $\underline{z}$ and $\underline{\theta}$), the new variables are defined at *points* located as shown in Figure A4.1a. As can be seen, the region between the center of the tissue (bottom end of the last cell) and the surface (front end of the first cell) is divided along the $\underline{z}$-axis into S increments with grid points being placed on the above two boundaries and on each interface demarcating the cells composing the

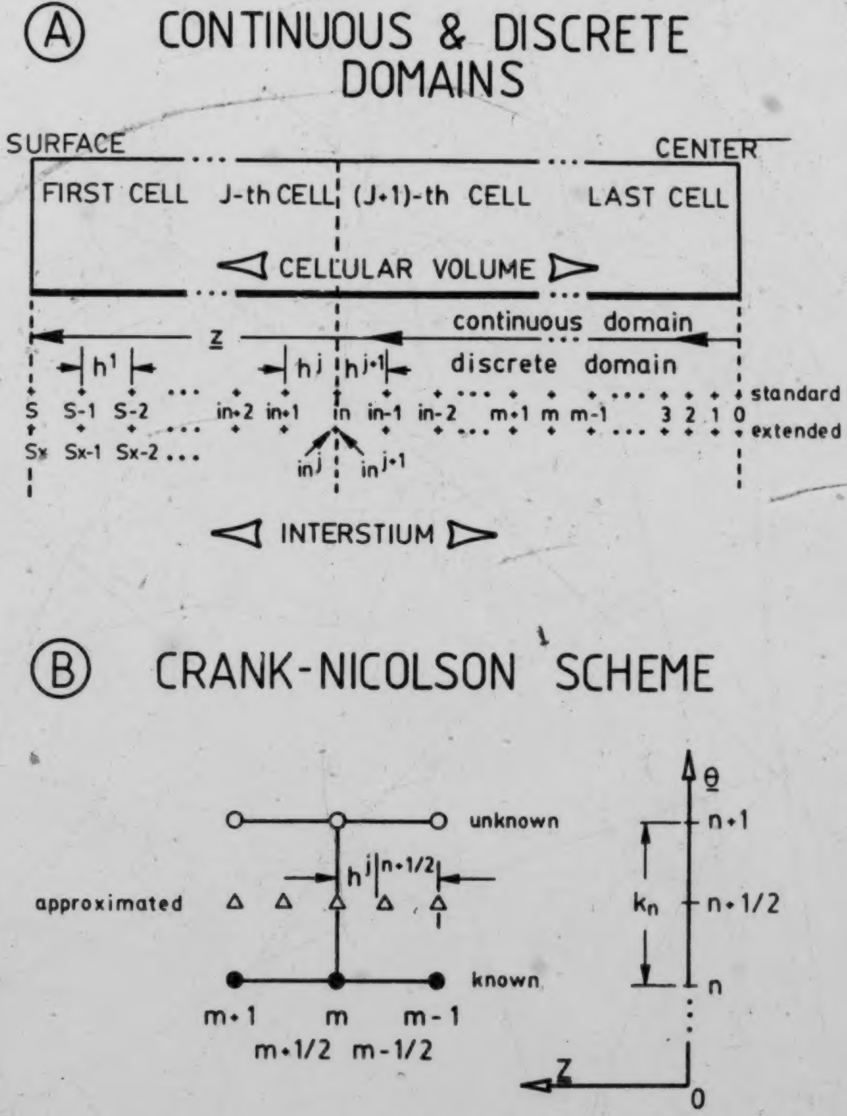

Ⓐ CONTINUOUS & DISCRETE DOMAINS
SURFACE
CENTER
FIRST CELL
J-th CELL
(J+1)-th CELL
LAST CELL
CELLULAR VOLUME
continuous domain
z
discrete domain
h¹
hʲ
hʲ⁺¹
S S-1 S-2 ... in+2 in+1 in in-1 in-2 m+1 m m-1 3 2 1 0
standard
Sx Sx-1 Sx-2 ...
extended
inʲ
inʲ⁺¹
INTERSTIUM
Ⓑ CRANK-NICOLSON SCHEME
unknown
approximated
known
hʲ|n+1/2
m+1 m m-1
m+1/2 m-1/2
θ
n+1
n+1/2
n
kₙ
z
0

Figure A4.1 Numerical solution of the extracellular continuity equations: (a) the continuous and discrete variable domains and (b) the Crank-Nicolson scheme.

column. This arrangement is necessary to allow the *space increments* (denoted h) to vary from cell to cell as we move from center to surface. The values of these steps are based on the length of the cells such that, for the j-th cell, the grid points located along its portion of the z-axis are separated by *equal* space increments defined as[28]:

$$h^{j} = \underline{\ell}^{j} \; / \; (\#grp - 1) \qquad [A4.79]$$

where #grp is the number of grid points in the cell (assumed constant and identical for all the cells). Relation A4.79 is important since it states that the space steps are a function not only of the distance along the extracellular continuum (since they vary from cell to cell [superscript j]) but also of time, since they are related to the current values of the *time dependent* lengths of the ECUCs (see equation A4.50). Note that it is through these variable increments that it will be possible, as part of the numerical procedure, to simulate the shrinkage process in the z direction.

Two space grids are necessary, in the present context, to describe the interstitium domain. The first one, the *standard space grid*, which has been introduced, consists of S increments running from the center to the surface of the column. The second one, which will be referred to as the

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[28] The superscript j will be used *explicitly* in sections 15.2, 15.3 and 15.4 to indicate quantities pertaining to the j-th cell. This is necessary since the numerical solution of the model equations (to be described in these sections) is approached using *discrete* variables and *point* values.

extended space grid, is an outcome of the necessity of monitoring the column behavior on a *cell-to-cell* basis (as discussed in section 8.2.2), due to the discontinuities created at the *interfaces* between cells. The extended space grid contains *supplementary points* at the *interfaces* such that the conditions there can be described with respect to *both sides,* i.e., if we refer for example to boundary conditions A4.76, A4.77 and A4.78, one can compute *at each interface, two values* of the same quantity, say $\underline{v}\blacksquare$, on the j-th side, $\underline{v}\blacksquare^{j}|_{in}$, and on the (j+1)-th cell side, $\underline{v}\blacksquare^{j+1}|_{in}$. Accordingly, transmembrane fluxes, production-depletion terms, etc. can be estimated at the interface *without crossing* the boundary, i.e. independently of the condition of the neighboring cell. One must realize however that these *double points* occupy the *same location* and that the transfer of the information pertaining to one cell to the other must be handled by the proper *interface boundary conditions.* The grid points *within* a given cell are located on this extended grid, equally spaced, as before, from front to back end, with increments h^{j}. Hence, the extended grid contains Sx = [(#c)(#grp)]-1 points, whereas the standard grid contains S = [(#c)(#grp)]-(#c) points with only 1 point per interface.

The time axis for its part is divided into increments of size k. For the approach that we propose to employ, it is desirable to increase the size of the time step as the solution progresses, a process referred to as *acceleration* *procedure* (Von Rosenberg, 1969). Consequently, the discrete points may be *unevenly spaced* along the $\underline{\theta}$-axis such that the value of time is given, after the n-th increment, by:

$$\underline{\theta}_n = \Sigma_t k_t \ ; \ t = 1,2,\ldots,n \qquad [A4.80]$$

The dependent variable, $\underline{C}i_l^j$, is now a function of two *discrete* independent variables, $\underline{z}$ and $\underline{\theta}$. It is therefore necessary to use the following symbolism to specify the value of $\underline{C}i_l^j$, and in fact, as we will see, of any variable, at a given point on the grids. Let the index m indicate position along the $\underline{z}$-axis, and the index n along the $\underline{\theta}$-axis. The value of $\underline{C}i_l^j$ at a distance m from the origin and at time $\underline{\theta}_n$ will be denoted $\underline{C}i_l^j|_m^n$. We must emphasize here the importance of the superscript j (or [j+1]), which indicates that the quantity under consideration belongs to the j-th ([j+1]-th) cell. This is particularly crucial, as we will see, for the treatment of the interfaces and other boundaries.

Among the numerous techniques available to solve parabolic PDEs, the *Crank-Nicolson scheme* has been selected as being the most advantageous procedure to ensure valid simulations, not only for its ease of implementation but also because it offers sufficient conditions of stability and convergence (the reader is referred to Carnahan *et al.* [1969], Von Rosenberg [1969] and Lapidus and Pinder [1982] for detailed discussions on methods for the numerical solution of partial differential equations). In the

Crank-Nicolson finite-difference method, the partial derivatives of the function of interest, i.e. $\underline{c}i_{i}{}^{j}$, with respect to distance, $\underline{z}$, and time, $\underline{\theta}$, are approximated as follows. Referring to Figure A4.1b, let us consider a *midpoint* of the time axis, that is, n+1/2. The values of the dependent variable, $\underline{c}i_{i}{}^{j}$, are computed about the point ($\underline{z}_{m}$, $\underline{\theta}_{n+1/2}$). The expressions comprising equation A4.34 are then rewritten in terms of the following finite-difference analogs (Carnahan *et al.*, 1969), for the j-th cell and the i-th species:

$$(\partial \underline{c}i_{i}{}^{j}/\partial \underline{\theta})|_{m}^{n+1/2} \cong (2/k_{n})(\underline{c}i_{i}{}^{j}|_{m}^{n+1/2} - \underline{c}i_{i}{}^{j}|_{m}^{n}) \qquad [A4.81]$$

and

$$(\partial \underline{c}i_{i}{}^{j}/\partial \underline{z})|_{m}^{n+1/2} \cong$$

$$.5\ (h^{j}|^{n+1/2})^{-1}\ (\underline{c}i_{i}{}^{j}|_{m+1}^{n+1/2} - \underline{c}i_{i}{}^{j}|_{m-1}^{n+1/2}) \qquad [A4.82]$$

where k_{n} and $h^{j}|^{n+1/2}$ are, respectively, the n-th time increment and the j-th space step at time $\underline{\theta}_{n+1/2}$. It is important to note that the latter increment is evaluated *at the midpoint on the time axis*. This is due, as discussed previously, to the fact that h^{j} is not constant and is related to the cell length, which changes with time as a function of the change in $\underline{c}i_{i}{}^{j}$. The space step must therefore be treated as a *nonlinear* term (see below).

The treatment of the first term of equation A4.34 follows an approach suggested by Von Rosenberg (1969), that is:

$$[\partial/\partial\underline{z}(\underline{B}\blacksquare_i{}^j\ \partial\underline{c}i_i{}^j/\partial\underline{z})]|_m^{n+1/2} \cong (h^j|^{n+1/2})^{-1} \cdot$$
$$[(\underline{B}\blacksquare_i{}^j\ \partial\underline{c}i_i{}^j/\partial\underline{z})|_{m+1/2}^{n+1/2} - (\underline{B}\blacksquare_i{}^j\ \partial\underline{c}i_i{}^j/\partial\underline{z})|_{m-1/2}^{n+1/2}] \qquad [A4.83]$$

which leads to, by making use of *central difference analogs* similar to equation A4.82 to rewrite the derivatives appearing on the right-hand side (RHS):

$$[\partial/\partial\underline{z}(\underline{B}\blacksquare_i{}^j\ \partial\underline{c}i_i{}^j/\partial\underline{z})]|_m^{n+1/2} \cong (h^j|^{n+1/2})^{-1} \cdot$$
$$\{[\underline{B}\blacksquare_i{}^j|_{m+1/2}^{n+1/2}\ (\underline{c}i_i{}^j|_{m+1}^{n+1/2} - \underline{c}i_i{}^j|_m^{n+1/2})/h^j|^{n+1/2}] -$$
$$[\underline{B}\blacksquare_i{}^j|_{m-1/2}^{n+1/2}\ (\underline{c}i_i{}^j|_m^{n+1/2} - \underline{c}i_i{}^j|_{m-1}^{n+1/2})/h^j|^{n+1/2}]\} \qquad [A4.84]$$

The terms involving values estimated at the n+1/2 time level are approximated by the *arithmetic average* of the finite difference analogs at the points $(\underline{z}_m,\underline{\theta}_n)$ and $(\underline{z}_m,\underline{\theta}_{n+1})$, that is, for example:

$$\underline{c}i_i{}^j|_m^{n+1/2} \cong .5\ (\underline{c}i_i{}^j|_m^{n+1} + \underline{c}i_i{}^j|_m^{n}) \qquad [A4.85]$$

which therefore yields, for equations A4.81, A4.82 and A4.84, respectively:

$$(\partial\underline{c}i_i{}^j/\partial\underline{\theta})|_m^{n+1/2} \cong (\underline{c}i_i{}^j|_m^{n+1} - \underline{c}i_i{}^j|_m^{n})/k_n \qquad [A4.86]$$

$$(\partial\underline{c}i_i{}^j/\partial\underline{z})|_m^{n+1/2} \cong .25\ (h^j|^{n+1/2})^{-1} \cdot$$
$$(\underline{c}i_i{}^j|_{m+1}^{n+1} - \underline{c}i_i{}^j|_{m-1}^{n+1} + \underline{c}i_i{}^j|_{m+1}^{n} - \underline{c}i_i{}^j|_{m-1}^{n}) \qquad [A4.87]$$

and

$$[\partial/\partial\underline{z}(\underline{B}\blacksquare_i{}^j\ \partial\underline{c}i_i{}^j/\partial\underline{z})]|_m^{n+1/2} \cong .5\ (h^j|^{n+1/2})^{-2} \cdot$$
$$[\underline{B}\blacksquare_i{}^j|_{m+1/2}^{n+1/2}\ \underline{c}i_i{}^j|_{m+1}^{n+1} - (\underline{B}\blacksquare_i{}^j|_{m+1/2}^{n+1/2} + \underline{B}\blacksquare_i{}^j|_{m-1/2}^{n+1/2})\underline{c}i_i{}^j|_m^{n+1} +$$
$$\underline{B}\blacksquare_i{}^j|_{m-1/2}^{n+1/2}\ \underline{c}i_i{}^j|_{m-1}^{n+1} + \underline{B}\blacksquare_i{}^j|_{m+1/2}^{n+1/2}\ \underline{c}i_i{}^j|_{m+1}^{n} -$$
$$(\underline{B}\blacksquare_i{}^j|_{m+1/2}^{n+1/2} + \underline{B}\blacksquare_i{}^j|_{m-1/2}^{n+1/2})\underline{c}i_i{}^j|_m^{n} + \underline{B}\blacksquare_i{}^j|_{m-1/2}^{n+1/2}\ \underline{c}i_i{}^j|_{m-1}^{n}]$$

[A4.88]

where the values of the corrected pseudo-binary coefficients are calculated as follows:

$$\underline{B}{\blacksquare}_i{}^j\big|_{m+1/2}^{n+1/2} \cong f[.5\,(\underline{C}i_i{}^j\big|_{m+1}^{n+1/2} + \underline{C}i_i{}^j\big|_{m}^{n+1/2}),\ \psi i^j\big|^{n+1/2}]$$

[A4.89]

and

$$\underline{B}{\blacksquare}_i{}^j\big|_{m-1/2}^{n+1/2} \cong f[.5\,(\underline{C}i_i{}^j\big|_{m}^{n+1/2} + \underline{C}i_i{}^j\big|_{m-1}^{n+1/2}),\ \psi i^j\big|^{n+1/2}]$$

[A4.90]

where f denotes a given function describing the dependence of. $\underline{B}{\blacksquare}_i{}^j$ on the local interstitial concentration and on the apparent interstitium diffusibility. One can observe that the expressions on the RHS are kept at the n+1/2 time level. This is to indicate that the diffusivities as well as other terms appearing in equation A4.34 require special treatment.

Relation A4.34, which can be written:

$$(\partial\underline{C}i_i{}^j/\partial\underline{\theta})\big|_m^{n+1/2} =$$

$$[\partial/\partial\underline{z}(\underline{B}{\blacksquare}_i{}^j\ \partial\underline{C}i_i{}^j/\partial\underline{z})]\big|_m^{n+1/2} - \underline{v}{\blacksquare}^j\big|_m^{n+1/2}(\partial\underline{C}i_i{}^j/\partial\underline{z})\big|_m^{n+1/2} -$$

$$[\partial\underline{v}{\blacksquare}^j/\partial\underline{z} + (\theta*/\underline{V}i^j)\partial\underline{V}i^j/\partial\theta]\big|_m^{n+1/2}\ \underline{C}i_i{}^j\big|_m^{n+1/2} - \underline{R}_i{}^j\big|_m^{n+1/2}$$

[A4.91]

contains, as mentioned previously, terms that are implicitly functions of $\underline{C}i_i{}^j$, namely: $\underline{B}{\blacksquare}_i{}^j$, $\underline{v}{\blacksquare}^j$, $[\partial\underline{v}{\blacksquare}^j/\partial\underline{z} + (\theta*/\underline{V}i^j)\partial\underline{V}i^j/\partial\theta]$ (which hereafter will be denoted for convenience by "[...]"), and $\underline{R}_i{}^j$. This poses a problem since the evaluation of these coefficients for n+1/2 requires knowledge of $\underline{c}i_{i}^{j}$ at the advanced time level, i.e. n+1 (through equation A4.85), which cannot be known unless equation A4.91 is solved, as we will see later on. This dilemma can however be overcome by making use of methods for solving *quasi-linear PDEs*, i.e. equations where the nonlinear terms are functions of the dependent variable *only* and not of its derivatives, as is the case for the above expression.

Thus, using a technique described by Von Rosenberg (1969) and referred to as *forward projection to half level in time*, the following procedure will be employed:

1. The value of $\underline{c}i_{i}^{j}|_{m}^{n+1/2}$ is determined *explicitly* from:

$$\underline{c}i_{i}^{j}|_{m}^{n+1/2} \cong \underline{c}i_{i}^{j}|_{m}^{n} + (k_{n}/2)(\partial \underline{c}i_{i}^{j}/\partial \underline{\theta})|_{m}^{n} \qquad [A4.92]$$

where the term $(\partial \underline{c}i_{i}^{j}/\partial \underline{\theta})|_{m}^{n}$ is evaluated from the finite-difference analog of equation A4.91 at the *n* time level.

2. This local estimate is then used to evaluate the nonlinear terms of equation A4.91.

3. Relation A4.91 is solved using an appropriate algorithm (to be discussed in section 15.2.3) to obtain an estimate of $\underline{c}i_{i}^{j}|_{m}^{n+1}$.

4. An iterative procedure is then applied to ensure convergence on an optimum value of $\underline{c}i_{i}^{j}|_{m}^{n+1/2}$ and accordingly of $\underline{c}i_{i}^{j}|_{m}^{n+1}$. In that case the next estimate is given by:

$$\underline{c}i_{i}^{j}|_{m}^{n+1/2} = .5\ (\underline{c}i_{i}^{j}|_{m}^{(n+1)} + \underline{c}i_{i}^{j}|_{m}^{n}) \qquad [A4.93]$$

where $\underline{C}i_i{}^j|_m^{(n+1)}$ is the value just obtained in step 3.

Equations A4.91 and A4.92 can now be developed and transformed into their finite-difference analogs. Inserting relations A4.86, A4.87 and A4.88 in equation A4.91 yields, on rearrangement:

$$\underline{C}i_i{}^j|_m^{n+1} - \underline{C}i_i{}^j|_m^n = A^j|^{n+1/2} \cdot$$

$$[\underline{B}\blacksquare_i{}^j|_{m+1/2}^{n+1/2}\ \underline{C}i_i{}^j|_{m+1}^{n+1} - (\underline{B}\blacksquare_i{}^j|_{m+1/2}^{n+1/2} + \underline{B}\blacksquare_i{}^j|_{m-1/2}^{n+1/2})\underline{C}i_i{}^j|_m^{n+1} +$$

$$\underline{B}\blacksquare_i{}^j|_{m-1/2}^{n+1/2}\ \underline{C}i_i{}^j|_{m-1}^{n+1}] + A^j|^{n+1/2} \cdot$$

$$[\underline{B}\blacksquare_i{}^j|_{m+1/2}^{n+1/2}\ \underline{C}i_i{}^j|_{m+1}^n - (\underline{B}\blacksquare_i{}^j|_{m+1/2}^{n+1/2} + \underline{B}\blacksquare_i{}^j|_{m-1/2}^{n+1/2})\underline{C}i_i{}^j|_m^n +$$

$$\underline{B}\blacksquare_i{}^j|_{m-1/2}^{n+1/2}\ \underline{C}i_i{}^j|_{m-1}^n] -$$

$$B^j|^{n+1/2}\ \underline{v}\blacksquare^j|_m^{n+1/2}(\underline{C}i_i{}^j|_{m+1}^{n+1} - \underline{C}i_i{}^j|_{m-1}^{n+1}) -$$

$$B^j|^{n+1/2}\ \underline{v}\blacksquare^j|_m^{n+1/2}(\underline{C}i_i{}^j|_{m+1}^n - \underline{C}i_i{}^j|_{m-1}^n) -$$

$$\Gamma|^n\ [\ldots]|_m^{n+1/2}\ \underline{C}i_i{}^j|_m^{n+1} - \Gamma|^n\ [\ldots]|_m^{n+1/2}\ \underline{C}i_i{}^j|_m^n -$$

$$2\Gamma|^n\ \underline{R}_i{}^j|_m^{n+1/2} \qquad [A4.94]$$

where we have introduced:

$$A^j|^{n+1/2} = .5\ k_n\ (h^j|^{n+1/2})^{-2} \qquad [A4.95a]$$

$$B^j|^{n+1/2} = .25\ k_n\ (h^j|^{n+1/2})^{-1} \qquad [A4.95b]$$

and

$$\Gamma|^n = k_n\ /\ 2 \qquad [A4.95c]$$

Regrouping all the terms involving values estimated at the advanced time level on the left-hand side (LHS) leads finally to:

$$-\mathit{CMM1}|_m^{n+1/2}\ \underline{C}i_i{}^j|_{m-1}^{n+1} + (1 + \mathit{CM}|_m^{n+1/2})\underline{C}i_i{}^j|_m^{n+1} -$$

$$\begin{aligned}
&\mathit{CMP1}\big|_m^{n+1/2}\ \underline{C}\mathrm{i}_i{}^j\big|_{m+1}^{n+1} = \\
&\mathit{CMM1}\big|_m^{n+1/2}\ \underline{C}\mathrm{i}_i{}^j\big|_{m-1}^{n} + \left(1 - \mathit{CM}\big|_m^{n+1/2}\right)\underline{C}\mathrm{i}_i{}^j\big|_m^n + \\
&\mathit{CMP1}\big|_m^{n+1/2}\ \underline{C}\mathrm{i}_i{}^j\big|_{m+1}^{n} - 2\Gamma\big|^n\ \underline{R}_i{}^j\big|_m^{n+1/2} \qquad [A4.96]
\end{aligned}$$

where

$$\mathit{CMM1}\big|_m^{n+1/2} = \left(A^j\big|^{n+1/2}\ \underline{\mathbf{B}}{\blacksquare}_i{}^j\big|_{m-1/2}^{n+1/2}\right) + \left(B^j\big|^{n+1/2}\ \underline{\mathrm{v}}{\blacksquare}^j\big|_m^{n+1/2}\right) \qquad [A4.97a]$$

$$\begin{aligned}
\mathit{CM}\big|_m^{n+1/2} = {} & A^j\big|^{n+1/2}\left(\underline{\mathbf{B}}{\blacksquare}_i{}^j\big|_{m+1/2}^{n+1/2} + \underline{\mathbf{B}}{\blacksquare}_i{}^j\big|_{m-1/2}^{n+1/2}\right) + \\
& \Gamma\big|^n\ [\ldots]\big|_m^{n+1/2} \qquad [A4.97b]
\end{aligned}$$

and

$$\mathit{CMP1}\big|_m^{n+1/2} = \left(A^j\big|^{n+1/2}\ \underline{\mathbf{B}}{\blacksquare}_i{}^j\big|_{m+1/2}^{n+1/2}\right) - \left(B^j\big|^{n+1/2}\ \underline{\mathrm{v}}{\blacksquare}^j\big|_m^{n+1/2}\right) \qquad [A4.97c]$$

Equation A4.96 is the *general finite-difference analog* of equation A4.91. As can be seen, it is *linear* in its coefficients since, through the process described previously (see equation A4.92), the values of the nonlinear terms appearing in *CMM1*, *CM* and *CMP1* can be evaluated from *known* values of the concentrations at the n time level. Equation A4.96 cannot however be solved explicitly since it contains 3 unknown concentrations on the LHS, i.e. the values of $\underline{C}\mathrm{i}_i{}^j$ at the advanced time level for $\underline{z}$ = m-1, m and m+1. We must therefore obtain supplementary relations to provide the information necessary to solve the analog. We will return to this point in section 15.2.3.

Applying the same derivation to equation A4.92, more precisely, to the partial derivative appearing in the second term, yields similarly, on rearrangement:

$$\underline{C}\mathrm{i}_i{}^{j}\big|_m^{n+1/2} = \mathit{CMM1}\big|_m^n\,\underline{C}\mathrm{i}_i{}^{j}\big|_{m-1}^n + (1 - \mathit{CM}\big|_m^n)\underline{C}\mathrm{i}_i{}^{j}\big|_m^n + \mathit{CMP1}\big|_m^n\,\underline{C}\mathrm{i}_i{}^{j}\big|_{m+1}^n - \Gamma\big|^n\,\underline{R}_i{}^{j}\big|_m^n \qquad [A4.98]$$

where the coefficients *CMM1*, *CM* and *CMP1* are given by relations A4.97a-c with the difference that the superscript n+1/2 is replaced by n. As can be seen, equation A4.98 is of *explicit* form, that is, it contains only 1 unknown on the LHS and consequently can be solved.

15.2.2 Finite-difference analogs at the boundaries

Equations A4.96 and A4.98 are valid for all space points *within* a cell except at the boundaries (see section 8.2.5), i.e. at the surface of the column, at the center and at each interface between 2 cells. For those points where the general equations are not valid, it is necessary to introduce the *boundary conditions* to generate the appropriate finite-difference analogs. Using the approach described by Von Rosenberg (1969) and Chinnan and Young (1977) and referring to the *standard* grid point arrangement shown in Figure A4.1a, these special relations are derived as follows[29].

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[29] It is important to remind the reader that we have adopted the convention that the grid point position index, m, increases from *center to surface*, i.e. from 0 to S (or Sx), whereas the cell position index, j, increases from *surface to center*, i.e. from 1 to #c (last cell, which will be represented for convenience by the superscript x).

Analogs at the surface

Two types of boundary condition can be encountered at the surface of the column, as given by relations A4.69 and A4.70. Let us consider first the most simple case, i.e. the condition when there is no resistance to mass transfer at the surface. This conditions states that *at all times* the concentration at the surface for any species is equal to the bath concentration, i.e. for the i-th species (from equation A4.78):

$$\underline{c}i_i^1|_s = \underline{c}b_i \qquad [A4.99]$$

Note that the superscript n is absent to indicate that equation A4.99 is valid at any time level. Substituting this relation in equations A4.96 and A4.98 for m = S-1 and j = 1 leads to:

$$\begin{aligned}-CSM2|_{s-1}^{n+1/2}\,\underline{c}i_i^1|_{s-2}^{n+1} + (1 + CSM1|_{s-1}^{n+1/2})\underline{c}i_i^1|_{s-1}^{n+1} = \\ CSM2|_{s-1}^{n+1/2}\,\underline{c}i_i^1|_{s-2}^{n} + (1 - CSM1|_{s-1}^{n+1/2})\underline{c}i_i^1|_{s-1}^{n} + \\ 2CB|_{s-1}^{n+1/2}\,\underline{c}b_i - 2\Gamma|^n\,\underline{R}_i^1|_{s-1}^{n+1/2}\end{aligned} \qquad [A4.100]$$

where we have replaced all occurrences of $\underline{c}i_i^1|_s$ by $\underline{c}b_i$. The nonlinear coefficients for their part are defined as:

$$CSM2|_{s-1}^{n+1/2} = (A^1|^{n+1/2}\,\underline{B}\blacksquare_i^1|_{s-1.5}^{n+1/2}) + (B^1|^{n+1/2}\,\underline{v}\blacksquare^1|_{s-1}^{n+1/2})$$

[A4.101a]

$$\begin{aligned}CSM1|_{s-1}^{n+1/2} = A^1|^{n+1/2}\,(\underline{B}\blacksquare_i^1|_{s-.5}^{n+1/2} + \underline{B}\blacksquare_i^1|_{s-1.5}^{n+1/2}) + \\ \Gamma|^n\,[\ldots]|_{s-1}^{n+1/2}\end{aligned} \qquad [A4.101b]$$

and

$$CB|_{s-1}^{n+1/2} = (A'|^{n+1/2}\,\underline{B}{\blacksquare}_i{}^1|_{s-.5}^{n+1/2}) - (B'|^{n+1/2}\,\underline{v}{\blacksquare}'|_{s-1}^{n+1/2}) \qquad [A4.101c]$$

Accordingly, equation A4.98 becomes, for m = S - 1:

$$\underline{c}i_i{}^j|_{s-1}^{n+1/2} = CSM2|_{s-1}^{n}\,\underline{c}i_i{}^1|_{s-2}^{n} + (1 - CSM1|_{s-1}^{n})\underline{c}i_i{}^1|_{s-1}^{n} + CB|_{s-1}^{n}\,\underline{c}b_i - \Gamma|^{n}\,\underline{R}_i{}^1|_{s-1}^{n} \qquad [A4.102]$$

The second type of condition describes the behavior of the surface concentration due to resistance to mass transfer, that is, for all time levels:

$$(\partial\underline{c}i_i{}^1/\partial\underline{z})|_s = [(\underline{v}{\blacksquare}'/\underline{B}{\blacksquare}_i{}^1)\underline{c}i_i{}^1]|_s - [K_i/(\underline{v}{\blacksquare}{*}\,\underline{B}{\blacksquare}_i{}^1|_s)](\underline{c}i_i{}^1|_s - \underline{c}b_i) \qquad [A4.103]$$

To specify the value of the partial derivative at the boundary, a *backward second-order-correct analog* to the derivative for m = S is used. This is to stay in agreement with the general analogs which, by virtue of the Crank-Nicolson scheme, are also second-order-correct. Hence, referring to Saul'yev (1964), the LHS of equation A4.103 is approximated by:

$$(\partial\underline{c}i_i{}^1/\partial\underline{z})|_s \cong (\underline{c}i_i{}^1|_{s-2} - 4\underline{c}i_i{}^1|_{s-1} + 3\underline{c}i_i{}^1|_s)/(2h') \qquad [A4.104]$$

Substituting this relation in the above boundary condition and solving for $\underline{c}i_i{}^1|_s$ leads, on rearrangement, to:

$$\underline{c}i_i{}^1|_s = [(T'\,K_i)/(\underline{B}{\blacksquare}_i{}^1\,E_i')]|_s\,\underline{c}b_i + (4/E_i'|_s)\underline{c}i_i{}^1|_{s-1} - (1/E_i'|_s)\underline{c}i_i{}^1|_{s-2} \qquad [A4.105]$$

where

$$E_i'|_s = 3 - (T'/\underline{B}^{\blacksquare}{}_i'|_s)(\underline{v}^{\blacksquare *}\ \underline{v}^{\blacksquare}{}'|_s - K_i) \qquad [A4.106a]$$

and

$$T' = 2h' / \underline{v}^{\blacksquare *} \qquad [A4.106b]$$

One can observe that as K_i tends to infinity so does $E_i'|_s$, which yields at the limit the condition described by relation A4.99. Equation A4.105 can therefore be used to approximate both types of conditions at the surface. Equations A4.96 and A4.98, for m = S - 1, are then given by relations A4.100 and A4.102 with the nonlinear coefficients now defined as:

$$\mathit{CSM2}|^{()}_{s-1} =$$
$$A'|^{()}\ [(E_i'|^{()}_s\ \underline{B}^{\blacksquare}{}_i'|^{()}_{s-1.5} - \underline{B}^{\blacksquare}{}_i'|^{()}_{s-.5})/E_i'|^{()}_s]$$
$$+ (B'|^{()}\ \underline{v}^{\blacksquare}{}'|^{()}_{s-1})[(E_i'|^{()}_s + 1)/E_i'|^{()}_s] \qquad [A4.107a]$$

$$\mathit{CSM1}|^{()}_{s-1} = (A'|^{()}/E_i'|^{()}_s) \cdot$$
$$[(E_i'|^{()}_s - 4)\underline{B}^{\blacksquare}{}_i'|^{()}_{s-.5} + E_i'|^{()}_s\ \underline{B}^{\blacksquare}{}_i'|^{()}_{s-1.5}] +$$
$$(4/E_i'|^{()}_s)(B'|^{()}\ \underline{v}^{\blacksquare}{}'|^{()}_{s-1}) + \Gamma|^n\ [\ldots]|^{()}_{s-1} \qquad [A4.107b]$$

and

$$\mathit{CB}|^{()}_{s-1} = [(T'|^{()}\ K_i)/(\underline{B}^{\blacksquare}{}_i'|^{()}_s\ E_i'|^{()}_s)] \cdot$$
$$[(A'|^{()}\ \underline{B}^{\blacksquare}{}_i'|^{()}_{s-.5}) - (B'|^{()}\ \underline{v}^{\blacksquare}{}'|^{()}_{s-1})] \qquad [A4.107c]$$

where the superscript () indicates that the time level can take the value of n+1/2 or n, as required. Note again that, as $E_i'|^{()}_s \rightarrow \infty$, the above coefficients become equivalent to the ones described by relations A4.101a, b and c, respectively.

Analogs at the center

The finite-difference analogs at the center depend on the geometry of the tissue under consideration. For a semi-infinite body, condition A4.72 (along with assumption A4.78) states that the concentration of species i at m = 0 is constant and equal to the initial tissue concentration for all time levels, that is:

$$\underline{C}i_i^x\big|_0 = \underline{C}i_{i0} \qquad \text{[A4.108]}$$

where the superscript x indicates a condition pertaining to the innermost cell of the column, i.e. the (#c)-th cell. Substituting this relation in equations A4.96 and A4.98 for m = 1 and j = x leads to:

$$\begin{aligned}(1 + C1\big|_1^{n+1/2})\underline{C}i_i^x\big|_1^{n+1} - C2\big|_1^{n+1/2}\,\underline{C}i_i^x\big|_2^{n+1} = {}\\ 2C0\big|_1^{n+1/2}\,\underline{C}i_{i0} + (1 - C1\big|_1^{n+1/2})\underline{C}i_i^x\big|_1^{n} + {}\\ C2\big|_1^{n+1/2}\,\underline{C}i_i^x\big|_2^{n} - 2\Gamma\big|^{n}\,\underline{R}_i^x\big|_1^{n+1/2}\end{aligned} \qquad \text{[A4.109]}$$

and

$$\begin{aligned}\underline{C}i_i^x\big|_1^{n+1/2} = C0\big|_1^{n}\,\underline{C}i_{i0} + (1 - C1\big|_1^{n})\underline{C}i_i^x\big|_1^{n} + {}\\ C2\big|_1^{n}\,\underline{C}i_i^x\big|_2^{n} - \Gamma\big|^{n}\,\underline{R}_i^x\big|_1^{n}\end{aligned} \qquad \text{[A4.110]}$$

where

$$C0\big|_1^{(\,)} = (A^x\big|^{(\,)}\,\underline{D}_{\blacksquare i}^{x}\big|_{.5}^{(\,)}) + (B^x\big|^{(\,)}\,\underline{v}_{\blacksquare}^{x}\big|_1^{(\,)}) \qquad \text{[A4.111a]}$$

$$C1\big|_1^{(\,)} = A^x\big|^{(\,)}(\underline{D}_{\blacksquare i}^{x}\big|_{1.5}^{(\,)} + \underline{D}_{\blacksquare i}^{x}\big|_{.5}^{(\,)}) + \Gamma\big|^{n}\,[\ldots]\big|_1^{(\,)} \qquad \text{[A4.111b]}$$

and

$$C2|_{1}^{(\,)} = (A^{x}|^{(\,)}\ \underline{B}\blacksquare_{i}^{x}|_{1.5}^{(\,)}) - (B^{x}|^{(\,)}\ \underline{v}\blacksquare^{x}|_{1}^{(\,)}) \qquad [A4.111c]$$

For an infinite slab, the boundary condition is as follows (see relation A4.73):

$$(\partial \underline{c}i_{i}^{x}/\partial \underline{z})|_{0} = 0 \qquad [A4.112]$$

To specify the value of the partial derivative at the boundary, a *forward second-order-correct analog* to the derivative for m = 0 is used. Hence, referring to Lapidus and Pinder (1982), relation A4.112 is approximated by:

$$(\partial \underline{c}i_{i}^{x}/\partial \underline{z})|_{0} \cong$$
$$(-\underline{c}i_{i}^{x}|_{2} + 4\underline{c}i_{i}^{x}|_{1} - 3\underline{c}i_{i}^{x}|_{0})/(2h^{x}) \qquad [A4.113]$$

This relation is then set equal to 0, yielding:

$$\underline{c}i_{i}^{x}|_{0} = (4/3)\underline{c}i_{i}^{x}|_{1} - (1/3)\underline{c}i_{i}^{x}|_{2} \qquad [A4.114]$$

Inserting equation A4.114 in the general analogs A4.96 and A4.98 for m = 1 and j = x leads, on rearrangement, to equations A4.109 and A4.110, with the difference that the coefficients *C0*, *C1* and *C2* are now given by:

$$C0|_{1}^{(\,)} = 0 \qquad [A4.115a]$$

$$C2|_{1}^{(\,)} = A^{x}|^{(\,)}\ [\underline{B}\blacksquare_{i}^{x}|_{1.5}^{(\,)} - (\underline{B}\blacksquare_{i}^{x}|_{.5}^{(\,)}/3)] -$$
$$(4/3)(B^{x}|^{(\,)}\ \underline{v}\blacksquare^{x}|_{1}^{(\,)}) \qquad [A4.115b]$$

and

$$C1|_{1}^{(\,)} = C2|_{1}^{(\,)} + \Gamma|^{n}\,[...]|_{1}^{(\,)} \qquad [A4.115c]$$

Treatment at the interface between two cells

As discussed in section 8.2.5, the extracellular equation of change at the interface between 2 cells is given by:

$$(\underline{A}i^{j}\ \underline{B}\blacksquare_{i}{}^{j}\ \partial\underline{C}i_{i}{}^{j}/\partial\underline{z})|_{in} =$$
$$(\underline{A}i^{j+1}\ \underline{B}\blacksquare_{i}{}^{j+1}\ \partial\underline{C}i_{i}{}^{j+1}/\partial\underline{z})|_{in} \qquad [A4.116]$$

where $\partial\underline{C}i_{i}{}^{j}/\partial\underline{z}$ and $\partial\underline{C}i_{i}{}^{j+1}/\partial\underline{z}$ are the gradients *at the interface* but evaluated in the j-th and (j+1)-th cells, respectively. Since this equation replaces the general finite-difference analogs (equations A4.96 and A4.98) at the interface, due to the discontinuties introduced by the different behavior of the neighboring cells, using the Crank-Nicolson scheme, we get for m = in, i.e. for any given interface:

$$\underline{A}i^{j}|_{in}^{n+1/2}\ \underline{B}\blacksquare_{i}{}^{j}|_{in}^{n+1/2}\ (\partial\underline{C}i_{i}{}^{j}/\partial\underline{z})|_{in}^{n+1/2} =$$
$$\underline{A}i^{j+1}|_{in}^{n+1/2}\ \underline{B}\blacksquare_{i}{}^{j+1}|_{in}^{n+1/2}\ (\partial\underline{C}i_{i}{}^{j+1}/\partial\underline{z})|_{in}^{n+1/2} \qquad [A4.117]$$

Since we cannot cross the interface to evaluate either gradient, the second-order-correct analogs introduced previously, i.e. equations A4.104 and A4.113, must be employed to approximate the above derivatives. Remembering that the cell position index increases from surface to center while the grid point position index increases from center to surface, we must employ the *forward* analog for the derivative pertaining to the j-th cell and the *backward* analog for the (j+1)-th derivative, i.e.:

$$(\partial \underline{c}i_{i}{}^{j}/\partial \underline{z})|_{in}^{n+1/2} \cong .5\ (h^{j}|^{n+1/2})^{-1}\ .$$

$$(-\underline{c}i_{i}{}^{j}|_{in+2}^{n+1/2} + 4\underline{c}i_{i}{}^{j}|_{in+1}^{n+1/2} - 3\underline{c}i_{i}{}^{j}|_{in}^{n+1/2}) \qquad [A4.118]$$

and

$$(\partial \underline{c}i_{i}{}^{j+1}/\partial \underline{z})|_{in}^{n+1/2} \cong .5\ (h^{j+1}|^{n+1/2})^{-1}\ .$$

$$(\underline{c}i_{i}{}^{j+1}|_{in-2}^{n+1/2} - 4\underline{c}i_{i}{}^{j+1}|_{in-1}^{n+1/2} + 3\underline{c}i_{i}{}^{j+1}|_{in}^{n+1/2}) \qquad [A4.119]$$

which yields, after inserting relations A4.118 and A4.119 in the boundary condition A4.117, on rearrangement:

$$(-\underline{c}i_{i}{}^{j}|_{in+2}^{n+1/2} + 4\underline{c}i_{i}{}^{j}|_{in+1}^{n+1/2} - 3\underline{c}i_{i}{}^{j}|_{in}^{n+1/2}) = \Psi_{i}{}^{j+}|_{in}^{n+1/2}\ .$$

$$(\underline{c}i_{i}{}^{j+1}|_{in-2}^{n+1/2} - 4\underline{c}i_{i}{}^{j+1}|_{in-1}^{n+1/2} + 3\underline{c}i_{i}{}^{j+1}|_{in}^{n+1/2}) \qquad [A4.120]$$

where

$$\Psi_{i}{}^{j+}|_{in}^{n+1/2} =$$

$$[(\underline{A}i^{j+1}\ \underline{B}\blacksquare_{i}{}^{j+1}\ h^{j})/(\underline{A}i^{j}\ \underline{B}\blacksquare_{i}{}^{j}\ h^{j+1})]|_{in}^{n+1/2} \qquad [A4.121]$$

The terms at the half-time level are replaced by the arithmetic average of the analogs at the time levels n and n+1 leading to, after regrouping all the advanced time level terms on the LHS:

$$-\Psi_{i}{}^{j+}|_{in}^{n+1/2}\ \underline{c}i_{i}{}^{j+1}|_{in-2}^{n+1} + 4\Psi_{i}{}^{j+}|_{in}^{n+1/2}\ \underline{c}i_{i}{}^{j+1}|_{in-1}^{n+1} -$$

$$(3 + 3\Psi_{i}{}^{j+}|_{in}^{n+1/2})\underline{c}i_{i}{}^{j+}|_{in}^{n+1} + 4\underline{c}i_{i}{}^{j}|_{in+1}^{n+1} - \underline{c}i_{i}{}^{j}|_{in+2}^{n+1} =$$

$$\Psi_{i}{}^{j+}|_{in}^{n+1/2}\ \underline{c}i_{i}{}^{j+1}|_{in-2}^{n} - 4\Psi_{i}{}^{j+}|_{in}^{n+1/2}\ \underline{c}i_{i}{}^{j+1}|_{in-1}^{n} +$$

$$(3 + 3\Psi_{i}{}^{j+}|_{in}^{n+1/2})\underline{c}i_{i}{}^{j+}|_{in}^{n} - 4\underline{c}i_{i}{}^{j}|_{in+1}^{n} + \underline{c}i_{i}{}^{j}|_{in+2}^{n}$$

$$[A4.122]$$

where we have made use of assumption A4.78, i.e.:

$$\underline{c}i_{i}^{\;j}\big|_{i\,n}^{(\,)} = \underline{c}i_{i}^{\;j+1}\big|_{i\,n}^{(\,)} = \underline{c}i_{i}^{\;j+}\big|_{i\,n}^{(\,)} \qquad [A4.123]$$

Equation A4.122 is the finite-difference analog to be used at any interface between 2 cells in place of relation A4.96. For its part, equation A4.98 is replaced by relation A4.120, which, once solved for $\underline{c}i_{i}^{\;j+}\big|_{i\,n}^{n+1/2}$ leads to:

$$\underline{c}i_{i}^{\;j+}\big|_{i\,n}^{n+1/2} = -(3 + 3\Psi_{i}^{\;j+}\big|_{i\,n}^{n+1/2})^{-1} \cdot$$
$$[\underline{c}i_{i}^{\;j}\big|_{i\,n+2}^{n+1/2} - 4\underline{c}i_{i}^{\;j}\big|_{i\,n+1}^{n+1/2} + \Psi_{i}^{\;j+}\big|_{i\,n}^{n+1/2} (\underline{c}i_{i}^{\;j+1}\big|_{i\,n-2}^{n+1/2} -$$
$$4\underline{c}i_{i}^{\;j+1}\big|_{i\,n-1}^{n+1/2})] \qquad [A4.124]$$

Relation A4.123 is crucial in the foregoing analysis since it permits the *mergence* of the information pertaining to each of the *two interface points* (which should normally be considered independently) into a unique quantity, therefore eliminating the need to use the *extended space grid* in the derivation.

15.2.3 Note on solving the system of linear finite-difference analogs

The set of linear equations obtained in sections 15.2.1 and 15.2.2, i.e. equations A4.96, A4.100, A4.109 and A4.122, is of the form:

$$c_1u_1 + d_1u_2 + 0 + \ldots + 0 = f_1 \qquad [A4.125a]$$

$$b_2u_1 + c_2u_2 + d_2u_3 + 0 + \ldots + 0 = f_2 \qquad [A4.125b]$$

$$0 + b_3u_2 + c_3u_3 + d_3u_4 + 0 + \ldots + 0 = f_3 \qquad [A4.125c]$$

$$\ldots$$

$$0 + \dots + 0 + b_m u_{m-1} + c_m u_m + d_m u_{m+1} + \\ 0 + \dots + 0 = f_m \qquad [A4.125d]$$

$$\dots$$

$$0 + \dots + 0 + a_{in} u_{in-2} + b_{in} u_{in-1} + c_{in} u_{in} + d_{in} u_{in+1} + \\ e_{in} u_{in+2} + 0 + \dots + 0 = f_{in} \qquad [A4.125e]$$

$$\dots$$

$$0 + \dots + 0 + b_{s-2} u_{s-3} + \\ c_{s-2} u_{s-2} + d_{s-2} u_{s-1} = f_{s-2} \qquad [A4.125f]$$

$$0 + \dots + 0 + b_{s-1} u_{s-2} + c_{s-1} u_{s-1} = f_{s-1} \qquad [A4.125g]$$

where the coefficients a, b, c, d and e represent the nonlinear terms described by equations A4.97a-c, A4.101a-c (or A4.107 a-c) and A4.111a-c (or A4.115a-c) and present in equations A4.122 and A4.124, whereas f regroups the right-hand sides of equations A4.96, A4.100, A4.109 and A4.122, each of which is a known quantity. The terms u refer to the concentrations at given grid points for the advanced time level n+1. The subscripts indicate the position on the discrete z-axis where the above quantities need to be evaluated. For example, equation A4.125a is analogous to equation A4.109 where m = 1 and $c_1 = (1 + C1|_1^{n+1/2})$ and $d_1 = -C2|_1^{n+1/2}$ while the entire RHS is f_1. Similarly, equations A4.125b, c, d and f represent the general analog A4.96 for m = 2, 3, m and S-2, respectively. Relation A4.125e describes the analog A4.122 and relation A4.125g, the condition when m = S-1.

As can be seen, the above relations define a *system of S-1 linear equations* containing S-1 unknown u's which can be readily solved by appropriate algorithms such as a Gaussian elimination method (Carnahan *et al.*, 1969). This becomes more obvious when the system is written in *matricial* form, i.e.:

$$[abcde]\,[u] = [f] \qquad [A4.126]$$

where [abcde], [u] and [f] are, respectively, the matrix of nonlinear coefficients, the column vector of unknown concentrations, and the column vector of RHS quantities. The special arrangement of equations A4.125a-g leads to a matrix called a *pentadiagonal band matrix* since only the *second lower*, *first lower*, *main*, *first upper* and *second upper* diagonals contain *non-zero* quantities. A method for solving such systems has been reported by Von Rosenberg (1969) and will be employed in the present work. Let us note at this level that the solution of the above system requires a minimum of information to lead to valid estimates of the extracellular concentrations. Due to the use of second-order-correct analogs, one can observe that the system can be set up only if at least 5 grid points are present in each cell, including the points at both ends. A safe number however would be 7 points per cell, which would ensure that all boundary derivatives are evaluated from *independent information*, i.e. from points that do not appear simultaneously in two boundary analogs.

15.3 Intracellular and geometrical equations of change

As we have indicated in section 15.2, the nonlinearity of the extracellular continuity equation (equation A4.34) is caused by the inherent dependence of the coefficients multiplying the dependent variable and its derivatives on the local values taken by this variable. A close look at the definition of these nonlinear terms reveals however that they are in fact not *direct functions* of the extracellular concentration but, more precisely, they depend on the *response* to extracellular changes of the cellular volumes in contact with the interstitium solution. For example, the terms $\partial \underline{v}_{\blacksquare}^{j}/\partial \underline{z}$, $\partial \underline{V}_i^{j}/\partial \theta$ and $\underline{R}_i^{j}$ are all dependent on the *transmembrane fluxes* generated when a nonequilibrium state exists between intra- and extracellular chemical potentials (see equations A4.37, A4.38, A4.44, A4.57 and A4.36).

These coefficients rely furthermore on the values taken by *geometrical* parameters, which have been shown to be directly functions of the behavior of the cellular volumes (see sections 8.2.4). One has only to consider the dependence of the corrected diffusivity, $\underline{D}_{\blacksquare i}^{j}$ (appearing in the first term of equation A4.34), on the local value of the apparent interstitium diffusibility, Ψ_i^{j} (through equation 8.79), to understand the necessity of monitoring continuously the cellular volume changes as affected by the diffusion phenomena occurring in the interstitium continuum. The solution of equation A4.34, which has been described in detail in the previous sections, is therefore *coupled* with solving the intracellular equations of change.

Referring to sections 15.1.3 and 15.1.4, one can observe that the intracellular equations of change (equations A4.43 and A4.44), as well as the geometrical relations A4.50 to A4.62, form a system of *ordinary differential equations (ODEs)* which need to be solved *simultaneously* with respect to *time*, the only independent variable appearing in these equations. Solutions are required at two crucial points on the time axis: firstly, at the *half-time level*, i.e. n+1/2, to allow for the evaluation of the nonlinear coefficients appearing in the analogs of equation A4.34 (following the special procedure described in section 15.2.1) and, secondly, at the *advanced time level*, n+1, to prepare the system for a new time increment.

The sequence of events leading to the solution of both systems of equations is as follows:

1. Estimates of the extracellular concentration for time level n+1/2, for all species and all *extended* space grid points, are evaluated explicitly from the conditions of the tissue at the n-th time level using the analogs of equation A4.92. Contrary to section 15.2.3, the *extended space grid* arrangement must be used to obtain the pertinent information for solving the *intracellular* equations of change since the situation is, in this case, completely different. Indeed, since the intracellular concentrations, which are decisive in the calculation of the local thermodynamic forces, vary from cell to cell, it then follows that *at the interfaces*,

although the *extracellular* concentrations are the same on both sides (through equation A4.123), the transmembrane fluxes will be *distinct*, leading, at these points, to different values for the nonlinear terms appearing in the analogs (if required). The expression "to be solved on a *cell-to-cell basis*" assumes here all its importance.

2. The system of ordinary differential equations describing the behavior of the cellular volumes is integrated for this half-time increment, i.e. $k_n/2$, leading to the cell conditions at n+1/2.

3. The values of the nonlinear coefficients appearing in the finite-difference equations can then be estimated from the known intra- and extracellular conditions at n+1/2.

4. The system is solved leading to estimates of the extracellular concentrations at n+1.

5. If these values are not satisfactory, i.e. the solution has not converged yet, new estimates of the extracellular concentrations at n+1/2 are evaluated (using relation A4.93), the time level counter is set back to n and one proceeds to step 2 until convergence is attained.

6. The conditions of the cells facing the new extracellular concentration profile are determined by integrating the system of ODEs for $k_n/2$, i.e. from the *converged* conditions at n+1/2 to n+1.

7. The time level counter is set to n = n+1, a new time increment is determined and one proceeds to step 1 until the final time of integration is reached.

As can be seen, the cellular response always follows the establishment of a new extracellular concentration profile. This is due to the intrinsic nature of numerical techniques which forces one to adopt a certain sequence in the operations leading to the desired solution. However if one selects the time increments, k_n, small enough, it is possible to picture the whole process as relatively simultaneous (which appears closer to the reality of the phenomenon). In fact, these small increments are necessary only at the *onset* of the dehydration process, i.e. when the difference between the bath conditions and the tissue conditions are most important. As dehydration progresses, the gradients tend to decrease, which allows one to increase k_n, an approach referred to as *acceleration procedure* (Von Rosenberg, 1969), i.e., after each complete cycle (steps 1 to 7):

$$k_{n+1} = \lambda\ k_n\ ;\ \lambda \geq 1 \qquad [A4.127]$$

where λ is an *acceleration factor*. This process is usually stopped when k_{n+1} reaches a maximum value, which then remains constant until the final time of integration is attained. This is to preserve the stability of the solution which could be jeopardized for large time increments. It should be pointed out however that, since the Crank-Nicolson scheme is *theoretically unconditionally stable*, the upper limit for k_{n+1} can be quite important. This limit can in fact be determined by observing the behavior of the solution as k_n increases (see section 9.1.1).

This brings us to the question of the method to be used to carry out the integration of the ODEs. As for solving partial differential equations, we must rely again on a numerical procedure since the system cannot be solved analytically. The method must perform the following functions:

1. Since the interval of integration is directly related to the time increment used in the Crank-Nicolson algorithm, the ODE solver should be able to maintain good accuracy for *variable time intervals*, i.e. it should lead to valid solutions for finely-spaced time points at the beginning, and should be able to adapt itself for increasing time intervals.
2. The method should minimize computational time, especially in view of the iterative procedure used in step 5 above.
3. Finally, the solver should handle efficiently *stiff differential equations*, i.e. equations which have greatly differing time constants, as is frequently the case for differential equations that represent the behavior of physical systems such as chemical reactions proceeding to equilibrium and discharging capacitors, phenomena which are quite analogous to our own system.

Among the numerous algorithms available to solve such ODEs (Carnahan *et al.*, 1969), a *predictor-corrector method*, more precisely, a *backward differentiation formula (BDF)* *method*, also called *stiff method of Gear*, was selected as being the most advantageous, taking into consideration the above constraints. The chosen algorithm is the version available in the IMSL library of statistical and mathematical routines (IMSL Library, 1980) and the reader is referred to the User's Manual for more details on subroutine DGEAR used in this work.

Let us point out here that, due to the availability of sophisticated "ready-to-use" packages, the solution of systems of ODEs does not pose a problem. This is not however the case for solving PDEs, which requires "custom-made" algorithms, tailored to fit the needs of each individual problem. This is why "canned" routines are scarce and are usually designed for standard-type problems, thus rendering their use limited.

We must still discuss how to tackle the integration, which needs to be performed in order to access the *net* transmembrane contribution in equations A4.43 and A4.44 ($\int$-symbol). As can be seen in Figure A4.1a, transmembrane fluxes can be evaluated solely *at* the *extended* space grid points along the interstitium continuum, since this is the only location where values of the extracellular concentrations are available. As transmembrane transport occurs *all along* the transfer area separating intra- and extracellular solutions, it is necessary to access the *profile* of transmembrane fluxes for the *entire* length of each cell in order to be able to sum the *local* contributions (on a cell-to-cell basis) to lead to the net transfer occurring across the plasmalemma of any given cell. A *one-dimensional quasi-cubic Hermite interpolation* procedure (subroutine IQHSCU, IMSL Library, 1980) was thus selected in conjunction with a *cubic spline quadrature (integrator)* (subroutine DCSQDU, IMSL Library, 1980) to compute the spline coefficients necessary to estimate the profile of transmembrane fluxes and to carry out the integration of those fluxes over the length of the cell under consideration.

The quasi-Hermite spline was preferred over other cubic spline interpolation procedures because of the way the algorithm handles the evaluation of the spline coefficients of the function at the available points. The procedure determines the slope at each grid point by utilizing the information at the point in question and at the two points on either side, with *no restriction* on the behavior of the second derivative at that location. This has the main advantage of ensuring that the interpolated values between 3 consecutive points having inexistent fluxes are truly null, and are not fluctuating around 0, as is the case with other algorithms which constrain the second derivative. It was found at that level that a minimum of 7 grid points per cell was necessary to represent accurately the profile of the fluxes.

15.4 Supplementary relations

15.4.1 Local values of the volume average velocity

The solution of equation A4.34 requires, concomitantly, the evaluation of the local gradients of the volume average velocity, $\partial\underline{v}^{\blacksquare j}/\partial\underline{z}$, and the determination of the average velocity per se, i.e. $\underline{v}^{\blacksquare j}$. Since we have access only to the former quantities along the interstitium, it becomes necessary to integrate these gradients to access the point values of $\underline{v}^{\blacksquare j}$. The process is as follows:

1. Starting at the surface, $\underline{v}^{\blacksquare\prime}|_{s}$ is computed through equation A4.71.
2. Then, using the quasi-Hermite spline procedure described in section 15.3, one generates the interpolative coefficients describing the profile of $\partial\underline{v}^{\blacksquare j}/\partial\underline{z}$ for the first cellular interstitium.
3. The local values of $\underline{v}^{\blacksquare\prime}$ (for the grid points pertaining to the first cell) are estimated *in sequence* using the cubic spline integrator. Note that, during the process, the calculated integral, Q, between, say, the lower limit z and upper limit z+ (z+ > z) is *subtracted* from the estimate of $\underline{v}^{\blacksquare\prime}$ at the *upper* limit of integration, i.e.:

$$\underline{v}^{\blacksquare\prime}|_{z} = \underline{v}^{\blacksquare\prime}|_{z+} - Q \qquad \text{[A4.128]}$$

since the integration is carried out in the *negative* direction on the $\underline{z}$-axis, i.e. from surface to center.

4. Once the velocity at the innermost grid point belonging

to the first cell has been evaluated, i.e. $\underline{v}_{\blacksquare}^{1}$ at the interface between cell 1 and cell 2, $\underline{v}_{\blacksquare}^{2}$ for the first grid point in cell 2 is computed using the boundary condition A4.76. The process then recommences from step 2 until the last cell has been covered.

15.4.2 Local values of apoplastic fluxes and overall mass variation

As can be seen in equations A4.67 and A4.68, the knowledge of the surface apoplastic fluxes, $\underline{N}_{i}^{1}|_{s}$ for i = 2,3,...,m, is necessary to follow the overall mass variation of the tissue. Referring to equations 8.73 and 8.76, one can observe that the values of $(\partial \underline{c}_{i}^{1}/\partial \underline{z})|_{s}$, i.e. the gradients of concentration at the surface, must be estimated to access the above interstitial fluxes. Since this information is of interest not only at the surface but for all the extended space grid points up to the center, as it characterizes the state of the mass transport phenomena in the interstitial volume, an interpolation procedure, analogous to the method introduced in section 15.3, is used to compute point values of the concentration gradients through a *cubic spline first derivative evaluator* (subroutine DCSEVU, IMSL Library, 1980) which employs the spline coefficients generated by the interpolative quasi-Hermite method to estimate the desired derivatives. This evaluation is performed as for the preceding ones, on a *cell-to-cell basis* since, as specified by the boundary condition A4.77, the gradients are not equal at the interfaces separating neighboring cells. Once these values are obtained, it becomes possible to calculate at *each extended space grid point*, for all time levels:

1. The *apoplastic diffusive flux*, $\underline{J}i\blacksquare_i^{\,j}$, from:

$$\underline{J}i\blacksquare_i^{\,j}|_m = -\underline{B}\blacksquare_i^{\,j}|_m\ (\partial\underline{C}i_i^{\,j}/\partial\underline{z})|_m \qquad [A4.129]$$

2. The *bulk apoplastic flux*, $\underline{F}i\blacksquare_i^{\,j}$, given by:

$$\underline{F}i\blacksquare_i^{\,j}|_m = \underline{C}i_i^{\,j}|_m\ \underline{v}\blacksquare^{j}|_m \qquad [A4.130]$$

and, finally,

3. The *net apoplastic flux*, $\underline{N}i\blacksquare_i^{\,j}|_m$, defined as:

$$\underline{N}i\blacksquare_i^{\,j}|_m = \underline{J}i\blacksquare_i^{\,j}|_m + \underline{F}i\blacksquare_i^{\,j}|_m \qquad [A4.131]$$

Equations A4.67 and A4.68, for their part, are handled as follows. As for the sequence adopted to solve the system of ODEs describing the behavior of the cellular volumes (see section 15.3), the mass variation of the tissue (overall changes and net intake or loss of diffusing species), represented in differential form by the aforementioned relations, are integrated at time levels n+1/2 and n+1 using, respectively, the *Euler's method* and the *modified Euler's method* (Carnahan *et al.*, 1969), that is:

1. For the half-time level, the new mass of the tissue is given by:

$$\underline{m}^t|^{n+1/2} = \underline{m}^t|^n + (k_n/2)(d\underline{m}^t/d\underline{\theta})|^n \qquad [A4.132]$$

where the derivative is evaluated using equation A4.67; whereas the net intake or loss of species i for that period is estimated from:

$$\underline{m}_i|^{n+1/2} = \underline{m}_i|^n + (k_n/2)(d\underline{m}_i/d\underline{\theta})|^n \qquad [A4.133]$$

where the derivative is computed using equation A4.68.

2. For the complete interval, that is, to obtain the conditions at n+1, the above quantities are computed from:

$$\underline{m}^t|^{n+1} = \underline{m}^t|^n + k_n(d\underline{m}^t/d\underline{\theta})|^{n+1/2} \qquad [A4.134]$$

and

$$\underline{m}_i|^{n+1} = \underline{m}_i|^n + k_n(d\underline{m}_i/d\underline{\theta})|^{n+1/2} \qquad [A4.135]$$

where we have applied the modified Euler's method. Note that the latter approach yields better estimates than if one had applied the Euler's approach twice, i.e. from n to n+1/2 and from n+1/2 to n+1. The reader is referred to Carnahan *et al*. (1969) for more details.

16. APPENDIX 5: LISTING OF PROGRAM *TISSUE*

16.1 Introduction

In the next pages the reader will find the complete listing of the program TISSUE which has been written to simulate the isothermal mass transport phenomena occurring in a hypothetical piece of plant storage tissue subjected to an osmotic solution (following the theoretical considerations described in Chapter 8 and Appendix 4). The program has been written in *FORTRAN 77* as implemented for the compiler developed by IBM Corporation (Anonymous, 1983). The reader is therefore referred to that reference for details concerning the statements appearing in the codes. Since the program includes extensive comments throughout, we will present here only the general structure of the algorithm.

16.2 General description of the program

The program is divided into 5 units which represent more or less the hierarchy in the calling sequence.

16.2.1 The main driver

The main driver controls the overall progress of the simulation. It calls sequentially all the routines necessary to perform the numerical integration of the model equations following the schemes described in section 15.2.1 (Crank-Nicolson algorithm) and section 15.3 (predictor-corrector method of Gear).

16.2.2 First level subroutines

These routines are called directly by the main driver to generate the information necessary to perform the numerical integration. This unit comprises 8 subroutines: INIT inputs the data and evaluates all the constants required by the program; PREP generates the information necessary to compute the non-linear coefficients appearing in the finite difference equations (see section 15.2); OUTPUT outputs the results of the calculations in a suitable form for analysis; NLCOEF computes the non-linear coefficients from the information transmitted by routine PREP; CSIHAF estimates the reduced interstitium concentrations for time level n+1/2; CELLCH controls the integration of the model ordinary differential equations using subroutine DGEAR (see section 15.3); MASSCH evaluates, for a given time interval, the overall mass variation of the piece (see section 15.4.2); and, finally, CSINP1 estimates the reduced interstitium concentrations for the advanced time level, i.e. n+1.

16.2.3 Second level subroutines

These routines are called for the most part by the first level subroutines to perform specialized tasks (as is the case for the third and fourth level subroutines). This unit is composed of 13 subroutines: CYLCEL evaluates the mensurations of the ECUC from the condition of the real tissue at full turgor (following the relations described in section 8.1.2); MODCOF generates the modified values of the membranes macroscopic permeability coefficients (see section 15.1.1); REFVAR computes the reference values used to transform the variables in dimensionless form; CNPAR initializes some parameters used by the Crank-Nicolson algorithm; DCFUNC computes the values of the pseudo-binary diffusivities as a function of the apoplast diffusibility and the interstitium concentrations (see equations A4.89, A4.90, 8.79 and 9.2); FLUXM and FLUXP evaluate, respectively, the transmembrane and symplastic fluxes (see section 15.1.1); CVFUNC evaluates the changes in cellular, interstitial and total volume of any ECUC (see equations A4.57, A4.58 and A4.44); VELOCT computes the volume average velocity from estimate of the local velocity gradient (see section 15.4.1); FLUXA evaluates the apoplastic fluxes (see equations A4.129, A4.130 and A4.131); GRFUNC and GRDUM are called by routine DGEAR (through routine CELLCH) to generate the information necessary to carry the numerical integration of the model ordinary differential equations (see section 15.3); finally, PENTAG contains the algorithm used to solve the system of linear finite-difference analogs (see section 15.2.3).

16.2.4 Third level subroutines

This unit contains 7 subroutines, which are as follows: BUILDM and BUILDP are used to generate the matrices of permeability coefficients required in the flux calculations; FGRADM and FGRADP evaluate, respectively, the differences in thermodynamic forces responsible for the observed fluxes across the plasmalemma and the plasmodesma pseudo-membrane (see section 15.1.1); TAFUNC evaluates the changes in membrane transfer areas (see equations A4.59, A4.60 and A4.62); GPFUNC similarly evaluates the changes in the ECUC mensurations (see section 15.1.4); and, finally, CCFUNC computes the changes in intracellular concentrations following equation A4.43.

16.2.5 Fourth level subroutines

This unit contains only one routine, ARMEAN, which is used to compute the average concentration of a given species across any membrane (see equations A4.16 and A4.24).

```
***********************************************************************
*                                                                     *
*  PROGRAM NAME   - TISSUE                                            *
*                                                                     *
*  LATEST VERSION    - JULY 2, 1985.                                  *
*                                                                     *
*  WRITTEN BY   - CHRISTIAN TOUPIN                                    *
*                 FOOD ENGINEERING GROUP                              *
*                 DEPARTMENT OF FOOD SCIENCE                          *
*                 UNIVERSITY OF ALBERTA                               *
*                 EDMONTON, ALBERTA.                                  *
*                                                                     *
*  PURPOSE  - THIS PROGRAM SIMULATES THE ISOTHERMAL MASS TRANSPORT    *
*             PHENOMENA OCCURRING IN A PLANT STORAGE TISSUE           *
*             SUBJECTED TO AN OSMOTIC SOLUTION.                       *
*                                                                     *
*  REMARKS  - THE TREATMENT IS LIMITED TO THE ANALYSIS OF MASS        *
*             TRANSFER OF NON-ELECTROLYTES IN A PIECE OF PLANT        *
*             TISSUE WHICH GEOMETRY IS SUCH THAT IT CAN BE            *
*             ASSIMILATED EITHER TO AN INFINITE SLAB OR TO A SEMI-    *
*             INFINITE MEDIUM.                                        *
*                                                                     *
*             AN EQUIVALENT CYLINDRICAL CELL APPROACH IS USED TO      *
*             MODEL AS CLOSE AS POSSIBLE THE AVERAGE PROPERTIES       *
*             AND CHARACTERISTICS OF THE REAL PLANT TISSUE.           *
*                                                                     *
*             BULK UNIDIRECTIONAL MASS TRANSFER ONLY IS CONSIDERED.   *
*             IT IS ASSUMED THAT THE TISSUE IS MACROSCOPICALLY        *
*             HOMOGENEOUS AND ISOTROPIC.                              *
*                                                                     *
*             THE REAL TISSUE IS ASSIMILATED TO A BUNDLE OF           *
*             COLUMNS MADE OF LINEAR ARRANGEMENTS OF UNIT             *
*             CYLINDRICAL CELLS. SINCE EACH COLUMN IS ASSUMED TO      *
*             BEHAVE IN A SIMILAR FASHION, ONLY ONE COLUMN IS         *
*             CONSIDERED FOR SIMULATION PURPOSE.                      *
*                                                                     *
*             THE MODEL HAS BEEN DESIGNED TO ALLOW NOT ONLY FOR       *
*             APOPLASTIC TRANSPORT BUT ALSO FOR SYMPLASTIC            *
*             TRANSPORT.                                              *
*                                                                     *
*             IT IS ASSUMED THAT BOTH TYPES ARE OF A PASSIVE          *
*             NATURE. IT ALSO SIMULATES THE INTERNAL VARIATIONS       *
*             OF INTRA AND EXTRACELLULAR VOLUMES, OF PLASMALEMMAL     *
*             AND PLASMODESMAL TRANSFER AREAS AND PERMITS TO          *
*             LOOK AT POSSIBLE SHRINKAGE OF THE WHOLE STRUCTURE.      *
*                                                                     *
*             LET US NOTE FINALLY, THAT THE OSMOTICUM AS WELL AS      *
*             THE CELLULAR AND EXTRACELLULAR SOLUTIONS ARE ASSUMED    *
*             TO BE DILUTE AND IDEAL SOLUTIONS. THIS IMPLIES,         *
*             AMONG OTHER THINGS, THAT THERE ARE NO INTERACTIONS      *
*             BETWEEN THE SPECIES DURING APOPLASTIC TRANSFER.         *
*                                                                     *
*  PROGRAMMING NOTES  - DETAILS AS TO THE VARIABLE DEFINITIONS        *
*                       AND DESCRIPTION OF THE ALGORITHM ARE          *
*             GIVEN THROUGHOUT THE PROGRAM TO FACILITATE ITS          *
*             UNDERSTANDING.                                          *
*                                                                     *
*  VARIABLES                                                          *
*                                                                     *
*             THE FOLLOWING CONSTANTS DETERMINE THE SIZE OF THE       *
*             ARRAYS TO BE USED IN THE PROGRAM:                       *
*                                                                     *
*              NSOL - MAXIMUM NUMBER OF SOLUTES ALLOWED. LAST         *
*                     SOLUTE IS ALWAYS THE TISSUE FICTITIOUS          *
*                     IMPERMEABLE SOLUTE (SEE SUBROUTINE INIT FOR     *
*                     DETAILS).                                       *
*                                                                     *
*            NGRPTS - NUMBER OF GRID POINTS ON THE EXTENDED SPACE     *
*                     GRID ASSUMING A MAXIMUM OF 10 GRID POINTS       *
*                     PER UNIT CYLINDRICAL CELL TO BE MODELLED.       *
*                     MINIMUM NUMBER OF GRID POINTS IS 7 PER CELL.    *
*                                                                     *
*            NCELLS - MAXIMUM NUMBER OF UNIT CYLINDRICAL CELLS        *
*                     PRESENT IN THE MODEL COLUMN.                    *
*                                                                     *
*             MAIN PROGRAM VARIABLES:                                 *
*                                                                     *
*            NLEVEL - CURRENT TIME LEVEL (DIMENSIONLESS).             *
*                                                                     *
*              TIME - CURRENT TIME, IN SEC.                           *
*                                                                     *
*             TSTEP - CURRENT INTEGRATION TIME STEP, IN SEC.          *
*                                                                     *
*             KSTEP - CURRENT INTEGRATION TIME STEP (IN REDUCED       *
*                     FORM).                                          *
*                                                                     *
*            LVLCNT - TIME LEVEL COUNTER WHICH INDICATES THE TIME     *
*                     LEVEL CORRESPONDING TO THE DATA TO BE USED      *
*                     IN THE CALCULATIONS.                            *
*                                                                     *
*              PCNT - COUNT OF OUTPUTS PERFORMED.                     *
*                                                                     *
*             PTEST - TEST TO DETERMINE IF OUTPUT IS DESIRED OR       *
*                     NOT.                                            *
*                                                                     *
*            ITECNT - ITERATION COUNTER FOR CRANK-NICOLSON            *
*                     INTEGRATION ALGORITHM.                          *
*                                                                     *
*            STAGEV - VECTOR OF FLAGS INDICATING THE DEHYDRATION      *
*                     STAGE REACHED BY EACH UNIT CELL IN THE MODEL    *
*                     COLUMN. IF SET TO 1, THE CELL IS STILL IN       *
*                     TURGOR. IF SET TO 2, THE CELL IS PLASMOLYSED    *
*                     AND WHEN SET TO 3, THE CELL CRITICAL VOLUME     *
*                     IS ATTAINED.                                    *
*                                                                     *
*              CSIM - MATRIX OF REDUCED INTERSTITIUM SOLUTE           *
*                     CONCENTRATIONS.                                 *
*                                                                     *
*              CSCM - MATRIX OF REDUCED CELL SOLUTE                   *
*                     CONCENTRATIONS.                                 *
*                                                                     *
*             CELLM - MATRIX OF REDUCED CELL MENSURATIONS.            *
*                                                                     *
*             MASSM - MATRIX OF REDUCED OVERALL MASS OF THE TISSUE    *
*                     AND OF REDUCED SOLUTE MASSES IN THE TISSUE.     *
*                                                                     *
*            THE ABOVE MATRICES CONTAIN DATA CORRESPONDING TO 3       *
*            TIME LEVELS: N, N + 1/2 AND N + 1.                       *
*                                                                     *
*              FLUXMM - MATRIX OF MODIFIED TRANSMEMBRANE FLUXES, IN  *
*                       GMOLE CM-3 SEC-1.                            *
*                                                                    *
*              FLUXPM - MATRIX OF MODIFIED PLASMODESMAL FLUXES, IN   *
*                       GMOLE CM-3 SEC-1.                            *
*                                                                    *
*                PDTM - MATRIX OF REDUCED VOLUMETRIC AND SOLUTE      *
*                       PRODUCTION-DEPLETION TERMS.                  *
*                                                                    *
*               CVTDM - MATRIX OF UNIT CELL VOLUME TIME DERIVATIVES, *
*                       IN SEC-1.                                    *
*                                                                    *
*              VELGRV - VECTOR OF REDUCED VOLUME AVERAGE VELOCITY    *
*                       GRADIENTS.                                   *
*                                                                    *
*                VELV - VECTOR OF REDUCED VOLUME AVERAGE VELOCITIES. *
*                                                                    *
*              FLUXAM - MATRIX OF REDUCED APOPLASTIC FLUXES.         *
*                                                                    *
*        GAMA, DELTA, - COEFFICIENTS USED IN CALCULATION OF          *
*        EPSIV          INTERSTITIUM CONCENTRATIONS (SEE SUBROUTINE  *
*                       NLCOEF FOR DETAILS, DIMENSIONLESS).          *
*                                                                    *
*  CRM2TV, CRM1TV, CBTV, CMM1TM, CMTM, CMP1TM, CIM2TM, CIM1TM,       *
*  CITM, CIP1TM, CIP2TM, COTV, C1TV, C2TV                            *
*                                                                    *
*                     - VECTORS AND MATRICES OF NON-LINEAR           *
*                       COEFFICIENTS OF THE FINITE DIFFERENCE        *
*                       EQUATIONS (SEE SUBROUTINE NLCOEF FOR         *
*                       DETAILS, DIMENSIONLESS).                     *
*                                                                    *
*              OLDCIM - MATRIX CONTAINING ESTIMATES OF REDUCED       *
*                       INTERSTITIUM CONCENTRATIONS AT THE N+1 TIME  *
*                       LEVEL.                                       *
*                                                                    *
*               CTEST - TEST VALUE USED TO CHECK CONVERGENCE IN      *
*                       CRANK-NICOLSON INTEGRATION ALGORITHM.        *
*                                                                    *
**********************************************************************

*     DEBUG UNIT(2), INIT
*     END DEBUG

      PROGRAM TISSUE

      INTEGER NSOL, NGRPTS, NCELLS

      PARAMETER(NSOL = 4, NGRPTS = 2500, NCELLS = 250)

*     COMMON VARIABLES.

      COMMON /C1/ SOLNUM, IMPNUM, PCONUM, RNUM, RNUMEX, CELNUM,
     &            GRPCEL, COLNUM
      COMMON /C3/ CSBOV(NSOL), CSCOV(NSOL), CSIOV(NSOL), CSRV(NSOL)
      COMMON /C6/ MWV(NSOL+1), TMASSO, MASSOV(NSOL)
      COMMON /C8/ GRTOL; GRSTEP, CNTOL, ITEMAX
      COMMON /C9/ HSTEPV(NCELLS), KSTEP
      COMMON /C11/ AVCDIF, TREF, VREF
      COMMON /C12/ CSIM(NSOL, 0:NGRPTS, 3), CSCM(NSOL, NCELLS, 3),
     &             CELLM(11, NCELLS, 3), MASSM(3, NSOL+1),
     &             STAGEV(NCELLS), CRITCV
      COMMON /C13/ NLEVEL, LVLCNT, GRPTI, CELLI

      REAL*4 CSBOV, CSCOV, CSIOV, CSRV, MWV, TMASSO, MASSOV, GRTOL,
     &       GRSTEP, CNTOL, HSTEPV, KSTEP, AVCDIF, TREF, VREF, CSIM,
     &       CSCM, CELLM, MASSM, CRITCV, NLEVEL

      INTEGER SOLNUM, IMPNUM, PCONUM, RNUM, RNUMEX, CELNUM, GRPCEL,
     &        COLNUM, ITEMAX, STAGEV, LVLCNT, GRPTI, CELLI

*     ROUTINE VARIABLES.

      REAL*4 TSTEPO, TSTEPM, TMAX, TPRINT, IRESFO,
     &       TIME, TSTEP, PTEST,
     &       FLUXMM(NSOL+1, 0:NGRPTS), FLUXPM(NSOL+1, NCELLS, 2),
     &       PDTM(NSOL+1, 0:NGRPTS), CVTDM(3, NCELLS),
     &       VELGRV(0:NGRPTS), VELV(0:NGRPTS),
     &       FLUXAM(NSOL+1, 0:NGRPTS, 3), GAMA, DELTA, EPSIV(NSOL),
     &       CRM2TV(NSOL), CRM1TV(NSOL), CBTV(NSOL),
     &       CMM1TM(NSOL, NGRPTS-(2*(NCELLS+1))),
     &       CMTM(NSOL, NGRPTS-(2*(NCELLS+1))),
     &       CMP1TM(NSOL, NGRPTS-(2*(NCELLS+1))),
     &       CIM2TM(NSOL, NCELLS-1), CIM1TM(NSOL, NCELLS-1),
     &       CITM(NSOL, NCELLS-1), CIP1TM(NSOL, NCELLS-1),
     &       CIP2TM(NSOL, NCELLS-1), COTV(NSOL), C1TV(NSOL),
     &       C2TV(NSOL), OLDCIM(NSOL, 0:NGRPTS), CTEST

      INTEGER PCNT, ITECNT

*     INPUT DATA AND EVALUATE ALL CONSTANTS TO BE USED THROUGHOUT
*     THE PROGRAM.

      CALL INIT(TSTEPO, TSTEPM, TMAX, TPRINT, IRESFO)

*     INITIALIZE COUNTERS AND DATA MATRICES.

      NLEVEL = 0.0
      TIME = 0.0
      TSTEP = TSTEPO
      KSTEP = TSTEP / TREF
      LVLCNT = 1
      PCNT = 0
      PTEST = 1.0
      ITECNT = 1

*     ALL CELLS ARE INITIALLY AT FULL TURGOR.

      DO 10 K = 1, CELNUM
         STAGEV(K) = 1
10    CONTINUE

*     PUT VECTORS OF INITIAL CONCENTRATIONS IN REDUCED FORM.

      DO 20 I = 1, SOLNUM
         CSBOV(I) = CSBOV(I) / CSRV(I)
         CSCOV(I) = CSCOV(I) / CSRV(I)
         CSIOV(I) = CSIOV(I) / CSRV(I)
20    CONTINUE
```

```
        DO 30 I = 1, SOLNUM
        DO 30 J = 0, RNUMEX
        DO 30 K = 1, 3
           CSIM(I, J, K) = CSIOV(I)
 30     CONTINUE

        DO 40 I = 1, SOLNUM
        DO 40 J = 1, CELNUM
        DO 40 K = 1, 3
           CSCM(I, J, K) = CSCOV(I)
 40     CONTINUE

        DO 50 J = 1, CELNUM
        DO 50 K = 1, 3
           CELLM(11, J, K) = IRESPO
           DO 50 I = 1, 10
              CELLM(I, J, K) = 1.0
 50     CONTINUE

        DO 60 I = 1, 3
           MASSM(I, 1) = 1.0
           DO 60 J = 1, SOLNUM
              MASSM(I, J+1) = MASSOV(J)
 60     CONTINUE

 80     CONTINUE

*       COMPUTE ALL NECESSARY INFORMATION TO EVALUATE NON-LINEAR
*       COEFFICIENTS OF FINITE DIFFERENCE EQUATIONS.

        CALL PREP(FLUXMM, FLUXPM, PDTM, CVTOM, VELGRV, VELV, FLUXAM)

*       OUTPUT CURRENT STATE OF THE TISSUE IF DESIRED.

        IF ((TIME .EQ. 0.0) .OR. (TIME .GE. PTEST)) THEN
          CALL OUTPUT(TIME, TSTEP, FLUXMM, FLUXPM, VELV, FLUXAM)
          PCNT = PCNT + 1
          PTEST = TPRINT * REAL(PCNT)
        END IF

*       TERMINATE INTEGRATION IF FINAL TIME LEVEL REACHED.

        IF (TIME .GE. TMAX) GO TO 999

*       EVALUATE NON-LINEAR COEFFICIENTS OF FINITE DIFFERENCE
*       EQUATIONS.

        CALL NLCOEF(VELGRV, VELV, CVTOM, GAMA, DELTA, EPSIV, CRM2TV,
     &              CRM1TV, CBTV, CMM1TM, CMTM, CMP1TM, CIM2TM,
     &              CIM1TM, CITM, CIP1TM, CIP2TM, C0TV, C1TV, C2TV)

        IF (LVLCNT .EQ. 1) THEN

*         ESTIMATE CONDITION OF THE TISSUE FOR TIME LEVEL N+1/2.

          LVLCNT = 2

*         ESTIMATE INTERSTITIUM SOLUTE CONCENTRATIONS.

          CALL CSINAF(PDTM, GAMA, DELTA, EPSIV, CRM2TV, CRM1TV, CBTV,
     &                CMM1TM, CMTM, CMP1TM, CIM2TM, CIM1TM, CITM,
     &                CIP1TM, CIP2TM, C0TV, C1TV, C2TV)

 110      CONTINUE

          DO 70 I = 1, SOLNUM
          DO 70 J = 0, RNUMEX
             OLDCIM(I, J) = CSIM(I, J, LVLCNT+1)
 70       CONTINUE

*         ESTIMATE CELL CONDITIONS AT THE N+1/2 TIME LEVEL.

          CALL CELLCH(*999)

*         ESTIMATE REDUCED MASS OF SOLUTES IN THE TISSUE AND REDUCED
*         OVERALL MASS OF THE TISSUE.

          CALL MASSCH(VELV, FLUXPM, FLUXAM)

          TIME = NLEVEL * TREF
          TSTEP = KSTEP-* TREF

          GO TO 80

        ELSE

*         ESTIMATE CONDITION OF THE TISSUE FOR TIME LEVEL N+1.

          LVLCNT = 3

*         ESTIMATE INTERSTITIUM SOLUTE CONCENTRATIONS.

          CALL CSINP1(PDTM, GAMA, DELTA, EPSIV, CRM2TV, CRM1TV, CBTV,
     &                CMM1TM, CMTM, CMP1TM, CIM2TM, CIM1TM, CITM,
     &                CIP1TM, CIP2TM, C0TV, C1TV, C2TV)

          DO 90 I = 1, SOLNUM
          DO 90 J = 0, RNUMEX

             CTEST = ABS((CSIM(I, J, LVLCNT) - OLDCIM(I, J)) /
     &               (OLDCIM(I, J) + 1.0))

             IF (CTEST .GT. CNTOL) THEN

               IF (ITECNT .GT. ITEMAX) THEN
                 WRITE(2, *)
                 WRITE(2, *) ' ********************************'
                 WRITE(2, *) ' *        >>> WARNING <<<       *'
                 WRITE(2, *) ' *   NO CONVERGENCE IN C-N      *'
                 WRITE(2, *) ' *          ALGORITHM.          *'
                 WRITE(2, *) ' ********************************'
                 GO TO 999
               END IF

               LVLCNT = 2

*              GET NEW ESTIMATES OF INTERSTITIUM CONCENTRATIONS AT
*              THE N+1/2 TIME LEVEL.

               DO 100 K = 1, SOLNUM
             DO 100 L = 0, RNUMEX
                CSIM(K, L, LVLCNT) = 0.5 * (CSIM(K, L, LVLCNT+1) +
  &                                   CSIM(K, L, LVLCNT-1))
100          CONTINUE

             NLEVEL = NLEVEL - (0.5 * KSTEP)
             ITECNT = ITECNT + 1
             GO TO 110

           END IF

80      CONTINUE

*       ESTIMATE CELL CONDITIONS AT THE N+1 TIME LEVEL.

        CALL CELLCH(*666)

*       ESTIMATE REDUCED MASS OF SOLUTES IN THE TISSUE AND REDUCED
*       OVERALL MASS OF THE TISSUE.

        CALL MASSCH(VELV, FLUXPM, FLUXAM)

        TIME = NLEVEL * TREF
        TSTEP = KSTEP * TREF

*       INCREASE REDUCED TIME STEP BY 10% UP TO A LIMIT OF
*       TSTEPM SEC.

        IF (TSTEP .LT. TSTEPM) KSTEP = 1.1 * KSTEP

*       TRANSFER, IN THE INFORMATION MATRICES, ALL ADVANCE TIME
*       LEVEL VALUES FROM THEIR N+1 TIME LEVEL POSITION TO THEIR
*       N TIME LEVEL POSITION.

        DO 120 J = 0, RNUMEX
        DO 120 I = 1, SOLNUM
           CSIM(I, J, LVLCNT-2) = CSIM(I, J, LVLCNT)
120     CONTINUE

        DO 130 J = 1, CELNUM
        DO 130 I = 1, SOLNUM
           CSCM(I, J, LVLCNT-2) = CSCM(I, J, LVLCNT)
130     CONTINUE

        DO 140 J = 1, CELNUM
        DO 140 I = 1, 11
           CELLM(I, J, LVLCNT-2) = CELLM(I, J, LVLCNT)
140     CONTINUE

        DO 150 J = 1, SOLNUM+1
           MASSM(LVLCNT-2, J) = MASSM(LVLCNT, J)
150     CONTINUE

        LVLCNT = 1
        ITECNT = 1

        GO TO 80

      END IF

666   CONTINUE
      STOP
      END

**********************************************************************
*                     FIRST LEVEL SUBROUTINES.                       *
**********************************************************************
*                                                                    *
*  ROUTINE NAME  - INIT                                              *
*                                                                    *
*  PURPOSE  - THIS SUBROUTINE INPUTS THE DATA AND EVALUATES ALL      *
*              THE CONSTANTS TO BE USED BY THE PROGRAM.              *
*                                                                    *
*  USAGE  - CALL INIT(TSTEPO, TSTEPM, TMAX, TPRINT, IRESFO)          *
*                                                                    *
*  ARGUMENTS AND VARIABLES                                           *
*                                                                    *
*          TSTEPO - INITIAL TIME STEP FOR CRANK-NICOLSON              *
*                   INTEGRATION ROUTINE, IN SEC.                     *
*                                                                    *
*          TSTEPM - MAXIMUM TIME STEP ALLOWED IN CRANK-NICOLSON       *
*                   INTEGRATION ROUTINE, IN SEC                      *
*                                                                    *
*            TMAX - FINAL TIME OF INTEGRATION OF CRANK-NICOLSON       *
*                   INTEGRATION ROUTINE, IN SEC.                     *
*                                                                    *
*          TPRINT - TIME INTERVAL AT WHICH PRINTOUT OF RESULTS OF    *
*                   PROGRAM CALCULATIONS IS DESIRED, IN SEC.         *
*                                                                    *
*          IRESFO - INITIAL RESISTANCE FACTOR OF THE INTERSTITIUM    *
*                   COMPLEX (DIMENSIONLESS).                         *
*                                                                    *
*          SOLNUM - TOTAL NUMBER OF DIFFERENT SOLUTES PRESENT        *
*                   IN OSMOTICUM (BATHING SOLUTION) AND CELL         *
*                   SOLUTION. NOTE THAT THE MODEL IS RESTRICTED      *
*                   TO NON-ELECTROLYTE SPECIES.                      *
*                                                                    *
*              >>> NOTE 1: IT IS ASSUMED THAT INITIALLY THE CELLS   *
*                           (AT FULL TURGOR) ARE IN EQUILIBRIUM      *
*                   WITH A DILUTE BINARY INTERSTITIUM SOLUTION.      *
*                   THE ONLY SOLUTE PRESENT AT THAT TIME IN THE      *
*                   TISSUE (IN CELL AS WELL AS IN INTERSTITIUM       *
*                   SOLUTION) IS CONSIDERED TO BE A FICTITIOUS       *
*                   IMPERMEABLE SPECIES WHICH IS RESPONSIBLE FOR     *
*                   THE INITIAL CELL AND INTERSTITIUM OSMOTIC        *
*                   PRESSURES (EQUIVALENT SUCROSE SOLUTION, SEE      *
*                   NOTE 4 BELOW).                                   *
*                                                                    *
*                   THEREFORE  SOLNUM = BATH SOLUTE(S) +             *
*                                     FICTITIOUS TISSUE SOLUTE.      *
*                                                                    *
*                   NOTE THAT ONE OF THE BATH SOLUTE(S) CAN BE       *
*                   THIS FICTITIOUS SPECIES.                         *
*                                                                    *
*          IMPNUM - TOTAL NUMBER OF DIFFERENT IMPERMEABLE SOLUTES    *
*                   PRESENT IN OSMOTICUM AND CELL SOLUTION (SEE      *
*                   NOTE 1 ABOVE).                                   *
*                                                                    *
*            PMVV - VECTOR OF THE PARTIAL MOLAR VOLUMES OF THE       *
*              SYSTEM SPECIES (WATER AND ALL SOLUTES), IN      *
*              CM3 GMOLE-1.                                    *
*                                                              *
*          >>> NOTE 2: THE INPUT ORDER: WATER(SOLVENT) THEN    *
*                      PERMEABLE SOLUTE(S) THEN IMPERMEABLE    *
*              SOLUTE(S). THE LAST ELEMENT(S) MUST BE THE      *
*              IMPERMEABLE SOLUTE(S) (BATH SPECIES FIRST,      *
*              CELL SPECIES LAST). THE FICTITIOUS SPECIES IS   *
*              THEREFORE ALWAYS THE LAST ELEMENT.              *
*                                                              *
*          MWV - VECTOR OF MOLECULAR WEIGHTS OF SYSTEM SPECIES, *
*              IN G GMOLE-1 (SEE NOTE 2 ABOVE).                *
*                                                              *
*        DIFCV - VECTOR OF PSEUDO-BINARY DIFFUSION COEFFICIENTS *
*              OF OSMOTICUM AND INTERSTITIUM SOLUTES IN        *
*              WATER AT INFINITE DILUTION, IN CM2 SEC-1.       *
*                                                              *
*         DEGV - VECTOR OF THE DEGREES OF THE SOLNUM           *
*              POLYNOMIALS USED TO MODEL THE CONCENTRATION     *
*              DEPENDENCE OF THE ABOVE DIFFUSIVITIES           *
*              (MAXIMUM DEGREE FOR ANY POLYNOMIAL IS 3).       *
*                                                              *
*          >>> NOTE 3: THE INPUT ORDER FOR ANY DATA RELATED    *
*                      TO THE SOLUTE(S) MUST FOLLOW THE SAME   *
*              INPUT POSITION SINCE THEY ARE ELEMENTS OF       *
*              VECTORS AND MATRICES. THEREFORE INPUT           *
*              PERMEABLE SOLUTE(S) FIRST THEN IMPERMEABLE      *
*              SOLUTE(S) WITH FICTITIOUS SPECIES AS LAST       *
*              ELEMENT.                                        *
*                                                              *
*       POLCOM - MATRIX OF THE POLYNOMIAL COEFFICIENTS. NOTE   *
*              THAT THE COEFFICIENTS MUST LEAD TO              *
*              DIFFUSIVITIES EXPRESSED IN CM2 SEC-1 USING      *
*              CONCENTRATIONS IN GMOLE CM-3.                   *
*                                                              *
*         TEMP - TEMPERATURE AT WHICH PROCESS OCCURS. ASSUMED  *
*              TO STAY CONSTANT, IN CELSIUS.                   *
*                                                              *
*            R - UNIVERSAL GAS CONSTANT, IN DYNE CM GMOLE-1    *
*              K-1.                                            *
*                                                              *
*        CSBOV - VECTOR OF INITIAL OSMOTICUM SOLUTE            *
*              CONCENTRATIONS, IN GMOLE CM-3 (SEE NOTE 1 AND   *
*              3 ABOVE). ASSUMED TO STAY CONSTANT. IF THE      *
*              FICTITIOUS SOLUTE IS NOT PRESENT IN THE BATH    *
*              INITIALLY, INPUT 0.                             *
*                                                              *
*          >>> NOTE 4: SINCE THE SOLUTIONS ARE ASSUMED TO BE   *
*                      IDEAL, THE CONCENTRATION DATA SHOULD    *
*              BE GIVEN IN A FORM CONSISTENT WITH VAN'T HOFF   *
*              EQUATION, I.E.:                                 *
*                                                              *
*                          CONC(I) = OP(I) / RT                *
*                                                              *
*              WHERE OP(I) IS THE SOLUTION OSMOTIC PRESSURE    *
*              DUE TO COMPONENT I AND RT IS THE UNIVERSAL      *
*              GAS CONSTANT MULTIPLIED BY THE TEMPERATURE OF   *
*              THE SYSTEM.                                     *
*                                                              *
*        SURFL - FLAG TO INDICATE IF THERE IS A RESISTANCE TO  *
*              MASS TRANSFER AT THE SURFACE OF THE TISSUE.     *
*              IF SURFL = 0, NO RESISTANCE, AMTRCV VECTOR      *
*              NEED NOT TO BE READ. IF SURFL = 1, VALUES OF    *
*              THE SURFACE MASS TRANSFER COEFFICIENTS MUST     *
*              BE PROVIDED.                                    *
*                                                              *
*       AMTRCV - VECTOR OF APPARENT PSEUDO-BINARY MASS TRANSFER *
*              COEFFICIENTS AT THE SURFACE OF THE TISSUE FOR   *
*              THE SYSTEM SOLUTES, IN CM SEC-1 (SEE NOTE 3     *
*              ABOVE).                                         *
*                                                              *
*       GEOMFL - FLAG TO INDICATE THE TYPE OF GEOMETRY         *
*              DESCRIBING THE TISSUE UNDER CONSIDERATION.      *
*              IF SET TO 1, THE TISSUE CAN BE ASSIMILATED TO   *
*              AN INFINITE SLAB; IF SET TO 0, IT IS A SEMI-    *
*              INFINITE MEDIUM.                                *
*                                                              *
*       TSAREA - WHEN THE TISSUE TO BE CONSIDERED CAN BE       *
*              ASSIMILATED TO AN INFINITE SLAB (GEOMFL = 1),   *
*              THE TOTAL SURFACE AREA OF TISSUE (TSAREA) IS    *
*              DEFINED AS THE AREA OF 1 OF THE FACES OF THE    *
*              TISSUE EXPOSED TO THE OSMOTICUM (THE EDGES ARE  *
*              NEGLECTED, IT IS ASSUMED THAT BOTH FACES ARE    *
*              SYMMETRICAL), IN CM2.                           *
*                                                              *
*              WHEN GEOMFL = 0 (SEMI-INFINITE MEDIUM), TSAREA  *
*              IS THE TOTAL SURFACE AREA EXPOSED TO THE BATH.  *
*                                                              *
*       THICKO - INITIAL TISSUE THICKNESS, IN CM. NOTE THAT THE *
*              TISSUE MUST BE AT LEAST 2 CELLS THICK.          *
*                                                              *
*              THE THICKNESS TO BE USED IN THE CALCULATIONS    *
*              SHOULD BE A CORRECTED VALUE TO TAKE INTO        *
*              ACCOUNT THE PERCENTAGE OF THE VOLUME OF THE     *
*              TISSUE WHICH IS "CUT CELLS" VOLUME. THIS IS     *
*              NECESSARY SINCE IT IS ASSUMED IN THE MODEL      *
*              THAT ALL CELLS ARE INTACT FROM SURFACE TO       *
*              CENTRE.                                         *
*                                                              *
*              FOR A SEMI-INFINITE MEDIUM, THE THICKNESS IS    *
*              DEFINED AS A FINITE DISTANCE FROM THE SURFACE   *
*              CHOSEN SUCH THAT THE CONDITIONS THERE REMAIN    *
*              CONSTANT AND EQUAL TO THE INITIAL VALUES        *
*              DURING THE ENTIRE DURATION OF THE DEHYDRATION   *
*              PROCESS.                                        *
*                                                              *
*       DIAMCO - INITIAL AVERAGE DIAMETER OF THE CELLS IN THE  *
*              TISSUE (SPHERE DELIMITED BY PLASMALEMMA),       *
*              IN CM.                                          *
*                                                              *
*        GTORT - GEOMETRICAL TORTUOSITY FACTOR (DIMENSIONLESS). *
*                                                              *
*  SPVFO, WVFO - INITIAL EXTRACELLULAR OPEN SPACE AND CELL WALL *
*              VOLUME FRACTIONS (DIMENSIONLESS).               *
*                                                              *
*       TMASSO - INITIAL MASS OF TISSUE, IN G.                 *
*                                                              *
*       MASSOV - VECTOR OF INITIAL MASS OF SYSTEM SOLUTES IN   *
*              THE TISSUE, IN G PER G TISSUE FRESH WEIGHT.     *
*              FOR SOLUTE(S) NOT PRESENT IN THE TISSUE         *
*                   INITIALLY BUT PRESENT IN BATH, INPUT 0 (SEE     *
*                   NOTE 1 AND 3 ABOVE).                            *
*                                                                   *
*           RNOVL - APPARENT RELATIVE NON-OSMOTIC CELL VOLUME,       *
*                   I.E., FRACTION OF TOTAL CELLULAR VOLUME THAT    *
*                   IS OSMOTICALLY INACTIVE. ASSUMED TO BE THE      *
*                   SAME FOR ALL CELLS AND TO STAY CONSTANT         *
*                   (DIMENSIONLESS).                                *
*                                                                   *
*          CRITCV - RELATIVE CELLULAR VOLUME AT WHICH THE CELLS     *
*                   REACH STAGE 3 OF DEHYDRATION, I.E., FRACTION    *
*                   OF THE INITIAL TOTAL CELLULAR VOLUME AT WHICH   *
*                   THE CELL MEMBRANE STARTS TO PULL THE CELL WALL  *
*                   (DIMENSIONLESS)                                 *
*                                                                   *
*           WRESF - CELL WALL RESISTANCE FACTOR (DIMENSIONLESS).    *
*                                                                   *
*          RPLASM - RATIO OF CELL MEMBRANE SURFACE AREA OCCUPIED    *
*                   BY PLASMODESMATA (DIMENSIONLESS).               *
*                                                                   *
*          EMODFL - FLAG TO INDICATE IF THE MODEL MUST INCLUDE, IN  *
*                   THE CALCULATIONS OF THE THERMODYNAMIC FORCES,   *
*                   THE PRESSURE DEPENDENCE OF THE CELL WALL        *
*                   ELASTIC MODULUS. IN THAT CASE, EMODFL IS SET    *
*                   TO 1. OTHERWISE, IT IS SET TO 0 AND THE CELL    *
*                   WALL ELASTIC MODULUS IS KEPT CONSTANT.          *
*                                                                   *
*                   THE PRESSURE DEPENDENCE OF THAT FACTOR IS       *
*                   ASSUMED TO BE OF THE FOLLOWING LINEAR FORM:     *
*                                                                   *
*                             EMOD = A * PC + B                     *
*                                                                   *
*                   WHERE A IS THE DERIVATIVE OF THE ELASTIC        *
*                   MODULUS WITH RESPECT TO THE CELL HYDROSTATIC    *
*                   PRESSURE (PC) AND B IS THE VALUE OF THE         *
*                   ELASTIC MODULUS AT INCIPIENT PLASMOLYSIS, I.E.  *
*                   WHEN PC = 0.                                    *
*                                                                   *
*          EMODCO - IF EMODFL = 0, CELL WALL ELASTIC MODULUS.       *
*                   VALUE TO BE KEPT CONSTANT, IN DYNE CM-2.        *
*                                                                   *
*                   IF EMODFL = 1, COEFFICIENTS OF THE LINEAR       *
*                   FUNCTION DESCRIBING THE PRESSURE DEPENDENCE     *
*                   OF THE ELASTIC MODULUS. 2 VALUES REQUIRED:      *
*                   A (DIMENSIONLESS) AND B IN DYNE CM-2.           *
*                                                                   *
*          COMPFV - VECTOR OF COMPLIANCE FACTOR VALUES RELATED TO   *
*                   EACH STAGE OF DEHYDRATION (DIMENSIONLESS).      *
*                                                                   *
*             PMV - VECTOR OF PERMEABILITY COEFFICIENTS (RELATED    *
*                   TO EACH PERMEABLE SPECIES PRESENT IN THE        *
*                   SYSTEM) OF EFFECTIVE CELL MEMBRANE,             *
*                   IN GMOLE DYNE-1 SEC-1.                          *
*                                                                   *
*               >>> NOTE 5: INPUT SHOULD BE IN VECTOR FORM SO AS    *
*                           TO PERMIT TRANSFORMATION INTO A FULL    *
*                   SYMMETRICAL MATRIX OF COEFFICIENTS. INPUT       *
*                   ORDER MUST BE IN AGREEMENT WITH PRECEDING       *
*                   INPUT OF PARTIAL MOLAR VOLUMES, I.E.:           *
*                                                                   *
*                   PMV(I), I = 1, .5 * (SOLNUM - IMPNUM + 1) *     *
*                                       (SOLNUM - IMPNUM + 2)       *
*                                                                   *
*                   EXAMPLE: PWW, PS1W, PS1S1, PS2W, PS2S1, PS2S2,  *
*                             ...., P(SOLNUM-IMPNUM)W, ....,        *
*                            P(SOLNUM-IMPNUM)(SOLNUM-IMPNUM).       *
*                                                                   *
*             PPV - VECTOR OF PERMEABILITY COEFFICIENTS (RELATED    *
*                   TO EACH PERMEABLE SPECIES PRESENT IN THE        *
*                   SYSTEM) OF EQUIVALENT PLASMODESMA MEMBRANE,     *
*                   IN GMOLE DYNE-1 SEC-1 (SEE NOTE 5 ABOVE).       *
*                   IN GMOLE DYNE-1 SEC-1 (SEE NOTE 5 ABOVE).       *
*                                                                   *
*               >>> NOTE 6: THE ABOVE PERMEABILITY COEFFICIENTS     *
*                           MUST BE THE VALUES AT THE TEMPERATURE   *
*                   OF THE SYSTEM, SINCE THERE IS NO PROVISION IN   *
*                   THE PROGRAM TO ACCOUNT FOR THEIR POSSIBLE       *
*                   TEMPERATURE DEPENDENCE.                         *
*                                                                   *
*           CSCOV - VECTOR OF INITIAL CELL SOLUTE CONCENTRATIONS,   *
*                                                                   *
*           CSCOV - VECTOR OF INITIAL CELL SOLUTE CONCENTRATIONS,   *
*                   IN GMOLE CM-3. FOR SOLUTE(S) NOT PRESENT IN     *
*                   CELL INITIALLY BUT PRESENT IN BATH, INPUT 0     *
*                   (SEE NOTE 1, 3 AND 4 ABOVE).                    *
*                                                                   *
*           CSIOV - VECTOR OF INITIAL INTERSTITIUM SOLUTE           *
*                   CONCENTRATIONS, IN GMOLE CM-3. FOR SOLUTE(S)    *
*                   NOT PRESENT IN INTERSTITIUM INITIALLY BUT       *
*                   PRESENT IN BATH, INPUT 0 (SEE NOTE 1, 3 AND 4   *
*                   ABOVE).                                         *
*                                                                   *
*           GRTOL - TOLERANCE FOR ERROR CONTROL IN GEARS            *
*                   INTEGRATION ROUTINE.                            *
*                                                                   *
*          GRSTEP - FRACTION OF ANY GIVEN TIME INTERVAL TO BE USED  *
*                   AS INITIAL INTEGRATION STEP IN GEARS            *
*                   INTEGRATION ROUTINE (SEE SUBROUTINE CELLCH      *
*                   FOR DETAILS).                                   *
*                                                                   *
*   CNTOL, ITEMAX - TOLERANCE FOR CONVERGENCE TEST IN ESTIMATION    *
*                   OF NON-LINEAR TERMS IN CRANK-NICOLSON           *
*                   ALGORITHM AND MAXIMUM NUMBER OF ITERATIONS      *
*                   ALLOWED TO CONVERGE.                            *
*                                                                   *
*          GRPCEL - NUMBER OF SPACE GRID POINTS PER CELL. DUE TO    *
*                   CERTAIN FEATURES OF THE ALGORITHM, THIS         *
*                   NUMBER MUST BE GREATER THAN OR EQUAL TO 7       *
*                   (MAXIMUM VALUE OF 10).                          *
*                                                                   *
*********************************************************************

*     DEBUG INIT, SUBTRACE
*     END DEBUG

      SUBROUTINE INIT(TSTEPO, TSTEPM, TMAX, TPRINT, IRESPO)

      INTEGER NSOL, NGRPTS, NCELLS
      PARAMETER(NSOL = 4, NGRPTS = 2500, NCELLS = 250)

*     ARGUMENTS.

      REAL*4 TSTEPO, TSTEPM, TMAX, TPRINT, IRESFO

*     COMMON VARIABLES.

      COMMON /C1/ SOLNUM, IMPNUM, PCONUM, RNUM, RNUMEX, CELNUM,
     &            GRPCEL, COLNUM
      COMMON /C2/ PMVV(NSOL+1), RT
      COMMON /C3/ CSBOV(NSOL), CSCOV(NSOL), CSIOV(NSOL), CSRV(NSOL)
      COMMON /C4/ CDIFCM(NSOL, 0:(NGRPTS*2)-NCELLS), SURFL,
     &            AMTRCV(NSOL)
      COMMON /C5/ SPVFO, WVFO, VOIDFO, VCO, VIO, VTO, AIO, ACMO,
     &            ACPO, LCO, RIO, RCO, RBO
      COMMON /C6/ MWV(NSOL+1), TMASSO, MASSOV(NSOL)
      COMMON /C7/ RNOVL, EMODFL, EMODCO(2), WRESF
      COMMON /C8/ GRTOL, GRSTEP, CNTOL, ITEMAX
      COMMON /C9/ HSTEPV(NCELLS), KSTEP
      COMMON /C12/ CSIM(NSOL, 0:NGRPTS, 3), CSCM(NSOL, NCELLS, 3),
     &             CELLM(11, NCELLS, 3), MASSM(3, NSOL+1),
     &             STAGEV(NCELLS), CRITCV
      COMMON /C14/ DIFCV(NSOL), DEGV(NSOL), POLCOM(NSOL, 0:4)
      COMMON /C15/ COMPFV(3)
      COMMON /C16/ GEOMFL

      REAL*4 PMVV, RT, CSBOV, CSCOV, CSIOV, CSRV, CDIFCM,
     &       AMTRCV, SPVFO, WVFO, VOIDFO, VCO, VIO, VTO, AIO, ACMO,
     &       ACPO, LCO, RIO, RCO, RBO, MWV, TMASSO, MASSOV, RNOVL,
     &       EMODCO, WRESF, GRTOL, GRSTEP, CNTOL, HSTEPV, KSTEP,
     &       CSIM, CSCM, CELLM, MASSM, CRITCV, DIFCV, POLCOM, COMPFV

      INTEGER SOLNUM, IMPNUM, PCONUM, RNUM, RNUMEX, CELNUM, GRPCEL,
     &        COLNUM, SURFL, EMODFL, ITEMAX, STAGEV, DEGV, GEOMFL

*     ROUTINE VARIABLES.

      REAL*4 TEMP, R, TSAREA, THICKO, DIAMCO, GTORT, RPLASM,
     &       PMV((NSOL+1)*(NSOL+2)/2),
     &       PPV((NSOL+1)*(NSOL+2)/2)

*     INPUT DATA.

*     SOLUTION CHARACTERISTICS.

      READ(1, *) SOLNUM, IMPNUM
      WRITE(2, *) ' SOLNUM, IMPNUM: ', SOLNUM, IMPNUM
      READ(1, *) (PMVV(I), I = 1, SOLNUM+1)
      WRITE(2, *) ' PMVV: ', (PMVV(I), I = 1, SOLNUM+1)
      READ(1, *) (MWV(I), I = 1, SOLNUM+1)
      WRITE(2, *) ' MWV: ', (MWV(I), I = 1, SOLNUM+1)
      DO 10 I = 1, SOLNUM
        READ(1, *) DIFCV(I), DEGV(I), (POLCOM(I, J), J = 0, DEGV(I))
        WRITE(2, *) ' DIFCV, DEGV, POLCOM: ', DIFCV(I), DEGV(I),
     &              (POLCOM(I, J), J = 0, DEGV(I))
10    CONTINUE

*     OSMOTICUM CHARACTERISTICS.

      READ(1, *) TEMP, R
      WRITE(2, *) ' TEMP, R: ', TEMP, R
      READ(1, *) (CSBOV(I), I = 1, SOLNUM)
      WRITE(2, *) ' CSBOV: ', (CSBOV(I), I = 1, SOLNUM)
      READ(1, *) SURFL
      IF (SURFL .EQ. 0) THEN
        WRITE(2, *) ' NO RESISTANCE AT THE SURFACE OF THE TISSUE.'
       ELSE
        READ(1, *) (AMTRCV(I), I = 1, SOLNUM)
        WRITE(2, *) ' AMTRCV: ', (AMTRCV(I), I = 1, SOLNUM)
      END IF

*     INITIAL TISSUE CHARACTERISTICS (FULL TURGOR TISSUE).

      READ(1, *) GEOMFL, TSAREA, THICKO, DIAMCO
      IF (GEOMFL .EQ. 1) THEN
        WRITE(2, *) ' INFINITE SLAB GEOMETRY CONSIDERED.'
       ELSE
        WRITE(2, *) ' SEMI-INFINITE MEDIUM GEOMETRY CONSIDERED.'
      END IF
      WRITE(2, *) ' TSAREA, THICKO, DIAMCO: ', TSAREA, THICKO,
     &            DIAMCO
      READ(1, *) GTORT, SPVFO, WVFO
      WRITE(2, *) ' GTORT, SPVFO, WVFO: ', GTORT, SPVFO, WVFO
      READ(1, *) TMASSO, (MASSOV(I), I = 1, SOLNUM)
      WRITE(2, *) ' TMASSO: ', TMASSO
      WRITE(2, *) ' MASSOV: ', (MASSOV(I), I = 1, SOLNUM)

*     CELL CHARACTERISTICS.

      READ(1, *) RNOVL, CRITCV, WRESF, RPLASM
      WRITE(2, *) ' RNOVL, CRITCV, WRESF, RPLASM: ', RNOVL,
     &            CRITCV, WRESF, RPLASM
      READ(1, *) EMODFL
      IF (EMODFL .EQ. 0) THEN
        READ(1, *) EMODCO(1)
        WRITE(2, *) ' ELASTIC MODULUS (KEPT CONSTANT): ', EMODCO(1)
       ELSE
        READ(1, *) (EMODCO(K), K = 1, 2)
        WRITE(2, *) ' LINEAR VARIATION OF ELASTIC MODULUS',
     &              ' CONSIDERED: ', (EMODCO(K), K = 1, 2)
      END IF
      READ(1, *) (COMPFV(K), K = 1, 3)
      WRITE(2, *) ' COMPLIANCE FACTOR VALUES: ',
     &            (COMPFV(K), K = 1, 3)
      PCONUM = ((SOLNUM - IMPNUM + 1) * (SOLNUM - IMPNUM + 2)) / 2
      READ(1, *) (PMV(K), K = 1, PCONUM)
      WRITE(2, *) ' PMV: ', (PMV(K), K = 1, PCONUM)
      READ(1, *) (PPV(K), K = 1, PCONUM)
      WRITE(2, *) ' PPV: ', (PPV(K), K = 1, PCONUM)
      READ(1, *) (CSCOV(I), I = 1, SOLNUM)
      WRITE(2, *) ' CSCOV: ', (CSCOV(I), I = 1, SOLNUM)

*     INTERSTITIUM CHARACTERISTICS.

      READ(1, *) (CSIOV(I), I = 1, SOLNUM)
      WRITE(2, *) ' CSIOV: ', (CSIOV(I), I = 1, SOLNUM)

*     PARAMETERS REQUIRED BY GEARS AND CRANK-NICOLSON ROUTINES.
```

```
      READ(1, *) GRTOL, GRSTEP, CNTOL, ITEMAX
      WRITE(2, *) ' GRTOL, GRSTEP, CNTOL, ITEMAX: ', GRTOL, GRSTEP,
     &            CNTOL, ITEMAX
      READ(1, *) GRPCEL
      WRITE(2, *) ' GRPCEL: ', GRPCEL
      READ(1, *) TSTEPO, TSTEPM, TMAX, TPRINT
      WRITE(2, *) ' TSTEPO, TSTEPM, TMAX, TPRINT: ', TSTEPO, TSTEPM,
     &            TMAX, TPRINT

*     GET INITIAL EQUIVALENT CYLINDRICAL UNIT CELL MENSURATIONS.

      CALL CYLCEL(DIAMCO, GTORT, RPLASM)

*     GET MODIFIED FORM OF PERMEABILITY COEFFICIENTS.

      CALL MODCOF(TEMP, R, PMV, PPV)

*     DEFINE SOME SUPPLEMENTARY REFERENCE VARIABLES FOR
*     TRANSFORMATION OF ORIGINAL DATA IN REDUCED (DIMENSIONLESS)
*     FORM.

      CALL REFVAR(IRESFO)

*     DEFINE SOME PARAMETERS TO BE USED BY CRANK-NICOLSON ALGORITHM.

      CALL CNPAR(TSAREA, THICKO)

      END

**********************************************************************
*                                                                    *
*  ROUTINE NAME  - PREP                                              *
*                                                                    *
*  PURPOSE  - THIS SUBROUTINE IS USED TO ESTIMATE THE INFORMATION    *
*             NECESSARY TO EVALUATE THE NON-LINEAR COEFFICIENTS      *
*             OF THE FINITE DIFFERENCE EQUATIONS.                    *
*                                                                    *
*  USAGE  - CALL PREP(FLUXMM, FLUXPM, PDTM, CVTDM, VELGRV, VELV,     *
*                     FLUXAM)                                        *
*                                                                    *
*  ARGUMENTS AND VARIABLES                                           *
*                                                                    *
*          FLUXMM - MATRIX OF MODIFIED TRANSMEMBRANE FLUXES, IN      *
*                   GMOLE CM-3 SEC-1.                                *
*                                                                    *
*          FLUXPM - MATRIX OF MODIFIED PLASMODESMAL FLUXES, IN       *
*                   GMOLE CM-3 SEC-1.                                *
*                                                                    *
*            PDTM - MATRIX OF REDUCED VOLUMETRIC AND SOLUTE          *
*                   PRODUCTION-DEPLETION TERMS.                      *
*                                                                    *
*           CVTDM - MATRIX OF UNIT CELL VOLUME TIME DERIVATIVES,     *
*                   IN SEC-1.                                        *
*                                                                    *
*          VELGRV - VECTOR OF REDUCED VOLUME AVERAGE VELOCITY        *
*                   GRADIENTS.                                       *
*                                                                    *
*            VELV - VECTOR OF REDUCED VOLUME AVERAGE VELOCITIES.     *
*                                                                    *
*          FLUXAM - MATRIX OF REDUCED APOPLASTIC FLUXES.             *
*                                                                    *
*           CELLI - CELL POSITION INDEX.                             *
*                                                                    *
*          GRPCNT - SPACE GRID POINT COUNTER.                        *
*                                                                    *
*           GRPTI - SPACE GRID POINT POSITION INDEX.                 *
*                                                                    *
*             PSI - VARIABLE USED IN CALCULATION OF REDUCED          *
*                   PRODUCTION-DEPLETION TERMS (DIMENSIONLESS).      *
*                                                                    *
*     SUM1, SUM2 - SUMMATION VARIABLES.                              *
*                                                                    *
**********************************************************************

*     DEBUG INIT, SUBTRACE
*     END DEBUG

      SUBROUTINE PREP(FLUXMM, FLUXPM, PDTM, CVTDM, VELGRV, VELV,
     &                FLUXAM)

      INTEGER NSOL, NGRPTS, NCELLS

      PARAMETER(NSOL = 4, NGRPTS = 2500, NCELLS = 250)

*     ARGUMENTS.

      REAL*4 FLUXMM(NSOL+1, 0:NGRPTS), FLUXPM(NSOL+1, NCELLS, 2),
     &       PDTM(NSOL+1, 0:NGRPTS), CVTDM(3, NCELLS),
     &       VELGRV(0:NGRPTS), VELV(0:NGRPTS),
     &       FLUXAM(NSOL+1, 0:NGRPTS, 3)

*     COMMON VARIABLES.

      COMMON /C1/ SOLNUM, IMPNUM, PCONUM, RNUM, RNUMEX, CELNUM,
     &            GRPCEL, COLNUM
      COMMON /C2/ PMVV(NSOL+1), RT
      COMMON /C3/ CSBOV(NSOL), CSCOV(NSOL), CSIOV(NSOL), CSRV(NSOL)
      COMMON /C5/ SPVFO, WVFO, VOIDFO, VCO, VIO, VTO, AIO, ACMO,
     &            ACPO, LCO, RIO, RCO, RBO
      COMMON /C8/ NSTEPV(NCELLS), KSTEP
      COMMON /C11/ AVCDIF, TREF, VREF
      COMMON /C12/ CSIM(NSOL, 0:NGRPTS, 3), CSCM(NSOL, NCELLS, 3),
     &             CELLM(11, NCELLS, 3), MASSM(3, NSOL+1),
     &             STAGEV(NCELLS), CRITCV
      COMMON /C13/ NLEVEL, LVLCNT, GRPTI, CELLI

      REAL*4 PMVV, RT, CSBOV, CSCOV, CSIOV, CSRV,
     &       SPVFO, WVFO, VOIDFO, VCO, VIO, VTO, AIO, ACMO,
     &       ACPO, LCO, RIO, RCO, RBO, NSTEPV, KSTEP, AVCDIF, TREF,
     &       VREF, CSIM, CSCM, CELLM, MASSM, CRITCV, NLEVEL

      INTEGER SOLNUM, IMPNUM, PCONUM, RNUM, RNUMEX, CELNUM, GRPCEL,
     &        COLNUM, STAGEV, LVLCNT, GRPTI, CELLI

*     ROUTINE VARIABLES.

      REAL*4 PSI, SUM1, SUM2
      INTEGER GRPCNT

*     COMPUTE CURRENT VALUES OF SPACE STEPS (FOR ALL CELLS).

      DO 10 J = 1, CELNUM
         HSTEPV(J) = CELLM(7, J, LVLCNT) / REAL(GRPCEL - 1)
10    CONTINUE

*     GET ESTIMATES OF THE LOCAL CORRECTED PSEUDO-BINARY DIFFUSION
*     COEFFICIENTS (FOR ALL SOLUTES).

      DO 20 I = 1, SOLNUM
      DO 20 J = 1, CELNUM
         CELLI = J
         CALL DCFUNC(I)
20    CONTINUE

*     EVALUATE LOCAL TRANSMEMBRANE FLUXES (FOR ALL SPECIES AND ALL
*     EXTENDED SPACE GRID POINTS, ON A CELL BY CELL BASIS).

      CELLI = 1
      GRPCNT = 1
      DO 30 J = RNUMEX, 0, -1
         GRPTI = J
         CALL FLUXM(FLUXMM)
         IF (GRPCNT .GE. GRPCEL) THEN
           CELLI = CELLI + 1
           GRPCNT = 1
          ELSE
           GRPCNT = GRPCNT + 1
         END IF
30    CONTINUE

*     EVALUATE, FOR EACH CELL, THE PLASMODESMAL FLUXES.

      DO 40 J = 1, CELNUM+1
         CELLI = J
         CALL FLUXP(FLUXPM)
40    CONTINUE

*     EVALUATE, ON A CELL BY CELL BASIS, THE LOCAL PRODUCTION-
*     DEPLETION TERMS (FOR ALL EXTENDED SPACE GRID POINTS).

      CELLI = 1
      GRPCNT = 1
      GRPTI = RNUMEX

70    CONTINUE

      PSI = ((1.0 - VOIDFO) * TREF * CELLM(5, CELLI, LVLCNT)) /
     &       (VOIDFO * CELLM(2, CELLI, LVLCNT))

80    CONTINUE

*     GET LOCAL SOLUTE PRODUCTION-DEPLETION TERM (FOR ALL SOLUTES).

      DO 50 I = 1, SOLNUM
         PDTM(I+1, GRPTI) = PSI * FLUXMM(I+1, GRPTI) / CSRV(I)
50    CONTINUE

*     GET LOCAL VOLUMETRIC PRODUCTION-DEPLETION TERM.

      SUM1 = 0.0
      DO 60 I = 1, SOLNUM+1
         SUM1 = SUM1 + (FLUXMM(I, GRPTI) * PMVV(I))
60    CONTINUE
      PDTM(1, GRPTI) = PSI * SUM1

      IF (GRPTI .LE. 0 ) THEN
        GO TO 90
       ELSE
        GRPTI = GRPTI - 1
      END IF

      IF (GRPCNT .GE. GRPCEL) THEN
        CELLI = CELLI + 1
        GRPCNT = 1
        GO TO 70
       ELSE
        GRPCNT = GRPCNT + 1
        GO TO 80
      END IF

90    CONTINUE

*     EVALUATE, FOR EACH CELL, THE CURRENT CELLULAR, INTERSTITIUM
*     AND TOTAL CELL VOLUME TIME DERIVATIVES.

      DO 100 J = 1, CELNUM
         CELLI = J
         CALL CVFUNC(FLUXMM, FLUXPM, CVTDM)
100   CONTINUE

*     EVALUATE THE LOCAL VOLUME AVERAGE VELOCITY GRADIENTS
*     (FOR ALL EXTENDED SPACE GRID POINTS, ON A CELL BY CELL BASIS).

      CELLI = CELNUM
      GRPCNT = 1
      DO 110 J = 0, RNUMEX
         VELGRV(J) = - (PDTM(1, J) + (TREF * CVTDM(2, CELLI) /
     &                    CELLM(2, CELLI, LVLCNT)))

         IF (GRPCNT .GE. GRPCEL) THEN
           CELLI = CELLI - 1
           GRPCNT = 1
          ELSE
           GRPCNT = GRPCNT + 1
         END IF

110   CONTINUE

*     EVALUATE THE REDUCED VOLUME AVERAGE VELOCITY AT THE SURFACE OF
*     THE TISSUE FROM THE MODEL COLUMN OVERALL VOLUME CHANGE.

      SUM1 = 0.0
      SUM2 = 0.0
      DO 120 J = 1, CELNUM
         SUM1 = SUM1 + CVTDM(3, J)
120   CONTINUE
```

```
      DO 130 I = 1, SOLNUM+1
         SUM2 = SUM2 + (FLUXPM(I, 1, 1) * PMVV(I))
130   CONTINUE

      VELV(RNUMEX) =((((1.0 - VOIDFO) * TREF * CELLM(8, 1, LVLCNT))
     &               / (VOIDFO * CELLM(4, 1, LVLCNT))) * SUM2) -
     &            ((TREF / (VOIDFO * CELLM(4, 1, LVLCNT))) * SUM1)

*     EVALUATE THE LOCAL REDUCED VOLUME AVERAGE VELOCITIES (ON A
*     CELL BY CELL BASIS, FOR ALL EXTENDED SPACE GRID POINTS).

      CALL VELOCT(VELGRV, VELV)

*     EVALUATE LOCAL VALUES OF REDUCED APOPLASTIC SOLUTE FLUXES.

      CALL FLUXA(VELV, FLUXAM)

      END

***********************************************************************
*                                                                     *
*  ROUTINE NAME  - OUTPUT                                             *
*                                                                     *
*  PURPOSE  - THIS SUBROUTINE OUTPUTS THE RESULTS OF THE              *
*             CALCULATIONS IN SUITABLE FORM FOR ANALYSIS.             *
*                                                                     *
*  USAGE  - CALL OUTPUT(TIME, TSTEP, FLUXMM, FLUXPM, VELV, FLUXAM)    *
*                                                                     *
*  ARGUMENTS AND VARIABLES                                            *
*                                                                     *
*            TIME - CURRENT TIME, IN SEC.                             *
*                                                                     *
*           TSTEP - CURRENT INTEGRATION TIME STEP, IN SEC             *
*                                                                     *
*          FLUXMM - MATRIX OF MODIFIED TRANSMEMBRANE FLUXES, IN       *
*                   GMOLE CM-3 SEC-1.                                 *
*                                                                     *
*          FLUXPM - MATRIX OF MODIFIED PLASMODESMAL FLUXES, IN        *
*                   GMOLE CM-3 SEC-1.                                 *
*                                                                     *
*            VELV - VECTOR OF REDUCED VOLUME AVERAGE VELOCITIES       *
*                                                                     *
*          FLUXAM - MATRIX OF REDUCED APOPLASTIC FLUXES.              *
*                                                                     *
*          STARTX - GRID POINT POSITION INDEX ON EXTENDED SPACE       *
*                   GRID.                                             *
*                                                                     *
*            SYMF - FACTOR TO TAKE INTO ACCOUNT POSSIBLE SYMMETRIC    *
*                   PROPERTIES OF THE GEOMETRY OF THE TISSUE UNDER    *
*                   CONSIDERATION.                                    *
*                                                                     *
*             SUM - SUMMATION VARIABLE.                               *
*                                                                     *
*          RTHICK - CURRENT RELATIVE THICKNESS OF THE TISSUE (AS      *
*                   COMPARED TO INITIAL THICKNESS).                   *
*                                                                     *
***********************************************************************

*     DEBUG  SUBTRACE
*     END DEBUG

      SUBROUTINE OUTPUT(TIME, TSTEP, FLUXMM, FLUXPM, VELV, FLUXAM)

      INTEGER NSOL, NGRPTS, NCELLS

      PARAMETER(NSOL = 4, NGRPTS = 2500, NCELLS = 250)

*     ARGUMENTS

      REAL*4 TIME, TSTEP, FLUXMM(NSOL+1, 0:NGRPTS),
     &       FLUXPM(NSOL+1, NCELLS, 2), VELV(0:NGRPTS),
     &       FLUXAM(NSOL+1, 0:NGRPTS, 3)

*     COMMON VARIABLES.

      COMMON /C1/ SOLNUM, IMPNUM, PCONUM, RNUM, RNUMEX, CELNUM,
     &            GRPCEL, COLNUM
      COMMON /C8/ HSTEPV(NCELLS), KSTEP
      COMMON /C4/ CDIFCM(NSOL, 0:(NGRPTS*2)-NCELLS), SURFL,
     &            AMTRCV(NSOL)
      COMMON /C12/ CSIM(NSOL, 0:NGRPTS, 3), CSCM(NSOL, NCELLS, 3),
     &             CELLM(11, NCELLS, 3), MASSM(3, NSOL+1),
     &             STAGEV(NCELLS), CRITCV
      COMMON /C13/ NLEVEL, LVLCNT, GRPTI, CELLI
      COMMON /C16/ GEOMFL

      REAL*4 CDIFCM, AMTRCV, HSTEPV, KSTEP, CSIM, CSCM, CELLM,
     &       MASSM, CRITCV, NLEVEL

      INTEGER SOLNUM, IMPNUM, PCONUM, RNUM, RNUMEX, CELNUM, GRPCEL,
     &        COLNUM, SURFL, STAGEV, LVLCNT, GRPTI, CELLI, GEOMFL

*     ROUTINE VARIABLES.

      REAL*4 SYMF, SUM, RTHICK

      INTEGER STARTX

*     OUTPUT CURRENT TIME AND CURRENT INTEGRATION TIME STEP, IN SEC.

      WRITE(2, 2000) TIME, TIME / 60.0, TSTEP
2000  FORMAT(//, '  CURRENT TIME: ', F8.2,' SEC, ', F7.2,' MIN.', /,
     &       '  CURRENT INTEGRATION TIME STEP (SEC): ', F7.4)

*     OUTPUT CURRENT REDUCED SOLUTE CONCENTRATIONS IN INTERSTITIUM.

      WRITE(2, *)
      WRITE(2, *)
      WRITE(2, *) ' EXTENDED SPACE GRID POSITION, REDUCED DISTANCE',
     &            ' FROM CENTRE,'
      WRITE(2, *) ' REDUCED SOLUTE CONCENTRATIONS:'

      DO 10 K = 1, CELNUM
         WRITE(2, 2010) K
2010     FORMAT(/, 4X, 'CELL POSITION INDEX: ', I5, /)
         SUM = 0.0
         DO 20 L = CELNUM, K, -1
            SUM = SUM + CELLM(7, L, LVLCNT)
```

```
20        CONTINUE
          STARTX = RNUMEX - ((K - 1) * GRPCEL)
          DO 10 J = 0, GRPCEL-1
             IF ((K .EQ. 1) .AND. (J .EQ. 0)) THEN
               WRITE(2, 2020) SUM, (CSIM(I, STARTX-J, LVLCNT),
     &                         I = 1, SOLNUM)
2020           FORMAT(11X, 'SURFACE', 3X, F8.4, 4(4X, F8.4))
              ELSE
               WRITE(2, 2030) STARTX-J, SUM,
     &                (CSIM(I, STARTX-J, LVLCNT), I = 1, SOLNUM)
2030           FORMAT(11X, I5, 5X, F8.4, 4(4X, F8.4))
             END IF
             SUM = SUM - HSTEPV(K)
10      CONTINUE

*       OUTPUT CURRENT VALUES OF REDUCED APOPLASTIC FLUXES.

        WRITE(2, *)
        WRITE(2, *)
        WRITE(2, *)' EXTENDED SPACE GRID POSITION, REDUCED APOPLASTIC'
     &            ,' FLUXES'
        WRITE(2, *) ' (DIFFUSIVE, CONVECTIVE AND NET), REDUCED',
     &              ' CORRECTED DIFFUSIVITY:'

        DO 30 I = 1, SOLNUM+1
           WRITE(2, 2040) I
2040       FORMAT(/, 4X, 'SPECIES INDEX: ', I3)
           DO 30 K = 1, CELNUM
              WRITE(2, 2050) K
2050          FORMAT(/, 4X, ' CELL POSITION INDEX: ', I5, /)
              STARTX = RNUMEX - ((K - 1) * GRPCEL)
              DO 30 J = 0, GRPCEL-1
                 IF (I .EQ. 1) THEN
                   IF ((K .EQ. 1) .AND. (J .EQ. 0)) THEN
                     WRITE(2, 2060) FLUXAM(I, STARTX-J, 3)
2060                 FORMAT(11X, 'SURFACE', 35X, F10.5)
                    ELSE
                     WRITE(2, 2070) STARTX-J, FLUXAM(I, STARTX-J, 3)
2070                 FORMAT(11X, I5, 37X, F10.5)
                   END IF
                 ELSE
                   IF ((K .EQ. 1) .AND. (J .EQ. 0)) THEN
                     WRITE(2, 2080) (FLUXAM(I, STARTX-J, L), L = 1, 3)
     &                         , CDIFCM(I-1, 2*(STARTX-J)-(CELNUM-K))
2080                 FORMAT(11X, 'SURFACE', 3X, 4(4X, F10.5))
                    ELSE
                     WRITE(2, 2090) STARTX-J,
     &                          (FLUXAM(I, STARTX-J, L), L = 1, 3),
     &                          CDIFCM(I-1, 2*(STARTX-J)-(CELNUM-K))
2090                 FORMAT(11X, I5, 5X, 4(4X, F10.5))
                   END IF
                 END IF
30      CONTINUE

*       OUTPUT CURRENT CELL REDUCED GEOMETRICAL CHARACTERISTICS,
*       REDUCED CONCENTRATIONS AND MODIFIED PLASMODESMAL FLUXES.

        WRITE(2, *)
        WRITE(2, *)
        WRITE(2, *) ' REDUCED GEOMETRICAL CHARACTERISTICS,',
     &              ' REDUCED SOLUTE CONCENTRATIONS,'
        WRITE(2, *) ' AND MODIFIED PLASMODESMAL FLUXES:'

        DO 40 K = 1, CELNUM
           WRITE (2, 2100) K, STAGEV(K)
2100       FORMAT(//, 4X, 'CELL POSITION INDEX: ',I5, 4X,
     &            'DEHYDRATION STAGE: ',I3, //)
           WRITE(2, 2110)
2110       FORMAT(3X, 'VC', 5X, 'VI', 5X, 'VT', 5X, 'AI', 5X, 'ACM',
     &            4X, 'ACP', 4X, 'LC', 5X, 'RI', 5X, 'RC', 5X, 'RB',
     &            4X, 'IRESF')
           WRITE(2, 2120) (CELLM(I, K, LVLCNT), I = 1, 11)
2120       FORMAT(11(2X, F5.3), /)
           WRITE(2, 2130)
2130       FORMAT(2X, 'SPECIES INDEX, CELL CONCENTRATION, ',
     &            'PLASMODESMAL FLUXES (FRONT END, BACK END):', /)
           DO 40 I = 1, SOLNUM+1
              IF (I .EQ. 1) THEN
                WRITE(2, 2140) I, (FLUXPM(I, K, L), L = 1, 2)
2140            FORMAT(8X, I3, 31X, 2(5X, F11.7))
               ELSE
                WRITE(2, 2150) I, CSCM(I-1, K, LVLCNT),
     &                         (FLUXPM(I, K, L), L = 1, 2)
2150            FORMAT(8X, I3, 12X, F8.4, 10X, 2(5X, F11.7))
              END IF
40       CONTINUE

*       OUTPUT CURRENT REDUCED VOLUME AVERAGE VELOCITIES AND MODIFIED
*       TRANSMEMBRANE FLUXES.

        WRITE(2, *)
        WRITE(2, *)
        WRITE(2, *) ' EXTENDED SPACE GRID POSITION, REDUCED VOLUME',
     &              ' AVERAGE VELOCITY,'
        WRITE(2, *) ' MODIFIED TRANSMEMBRANE FLUXES:'

        DO 50 K = 1, CELNUM
           WRITE(2, 2160) K
2160       FORMAT(/, 4X, 'CELL POSITION INDEX: ', I5, /)
           WRITE(2, 2170)
2170       FORMAT(22X, 'VELOCITY', 7X, 'TRANSMEMBRANE FLUXES', /,
     &            38X, '(SOLVENT THEN SOLUTE(S):', /)
           STARTX = RNUMEX - ((K - 1) * GRPCEL)
           DO 50 J = 0, GRPCEL-1
              IF ((K .EQ. 1) .AND. (J .EQ. 0)) THEN
                WRITE(2, 2180) VELV(STARTX-J),
     &                (FLUXMM(I, STARTX-J), I = 1, SOLNUM+1)
2180            FORMAT(11X, 'SURFACE', 3X, F10.4, 3X, 4(5X, F11.7))
               ELSE
                WRITE(2, 2190) STARTX-J, VELV(STARTX-J),
     &                (FLUXMM(I, STARTX-J), I = 1, SOLNUM+1)
2190            FORMAT(11X, I5, 5X, F10.4, 3X, 4(5X, F11.7))
              END IF
50      CONTINUE

*       OUTPUT CURRENT REDUCED MASS OF THE TISSUE AND OF THE SOLUTES
*       IN THE TISSUE. OUTPUT CURRENT REDUCED TISSUE THICKNESS AND
*       RELATIVE CHANGE FROM INITIAL VALUE.

        WRITE(2, *)
```

```
      WRITE(2, *)
      WRITE(2, 2200) MASSM(LVLCNT, 1)
2200  FORMAT('  CURRENT REDUCED MASS OF THE TISSUE: ', F8.4, /)
      WRITE(2, *) ' SOLUTE INDEX, REDUCED MASS OF SOLUTE IN TISSUE:'
      WRITE(2, *)

      DO 80 I = 1, SOLNUM
         WRITE(2, 2210) I, MASSM(LVLCNT, I+1)
2210     FORMAT(4X, I3, 5X, F7.4)
80    CONTINUE

      WRITE(2, *)
      WRITE(2, *)

      SUM = 0.0
      DO 70 J = 1, CELNUM
         SUM = SUM + CELLM(7, J, LVLCNT)
70    CONTINUE
      IF (GEOMFL .EQ. 1) THEN
        SYMF = 2.0
       ELSE
        SYMF = 1.0
      END IF
      WRITE(2, 2220) SUM * SYMF
2220  FORMAT('  CURRENT REDUCED THICKNESS OF TISSUE: ', F8.4)
      RTHICK = SUM / REAL(CELNUM)
      WRITE(2, 2230) RTHICK
2230  FORMAT('  CURRENT RELATIVE THICKNESS OF TISSUE: ', F8.4)

      END

*******************************************************************
*                                                                 *
*  ROUTINE NAME  - NLCOEF                                         *
*                                                                 *
*  PURPOSE   - THIS SUBROUTINE EVALUATES THE NON-LINEAR COEFFICIENTS*
*              THAT APPEAR IN THE FINITE DIFFERENCE EQUATIONS AT   *
*              THE N-TH TIME LEVEL AND FOR ALL SOLUTES.            *
*                                                                 *
*  USAGE  - CALL NLCOEF(VELGRV, VELV, CVTDM, GAMA, DELTA, EPSIV,  *
*                       CRM2TV, CRM1TV, CBTV, CMM1TM, CMTM, CMP1TM,*
*                       CIM2TM, CIM1TM, CITM, CIP1TM, CIP2TM, COTV,*
*                       C1TV, C2TV)                                *
*                                                                 *
*  ARGUMENTS AND VARIABLES                                        *
*                                                                 *
*             VELGRV - VECTOR OF REDUCED VOLUME AVERAGE VELOCITY   *
*                      GRADIENTS.                                  *
*                                                                 *
*               VELV - VECTOR OF REDUCED VOLUME AVERAGE VELOCITIES.*
*                                                                 *
*              CVTDM - MATRIX OF UNIT CELL VOLUME TIME DERIVATIVES,*
*                      IN SEC-1.                                   *
*                                                                 *
*       GAMA, DELTA, - COEFFICIENTS USED IN CALCULATION OF SOME    *
*       EPSIV          NON-LINEAR TERMS (DIMENSIONLESS).           *
*                                                                 *
*    CRM2TV, CRM1TV, - VECTORS OF NON-LINEAR COEFFICIENTS OF THE   *
*    CBTV              (R-1)-TH SPACE GRID POINT FINITE DIFFERENCE *
*                      EQUATIONS (DIMENSIONLESS).                  *
*                                                                 *
*      CMM1TM, CMTM, - MATRICES OF NON-LINEAR COEFFICIENTS OF THE  *
*      CMP1TM          M-TH SPACE GRID POINT FINITE DIFFERENCE     *
*                      EQUATIONS (DIMENSIONLESS, 2 <= M <= R-2,    *
*                      M IS NOT AN INTERFACE POINT).               *
*                                                                 *
*    CIM2TM, CIM1TM, - MATRICES OF NON-LINEAR COEFFICIENTS OF THE  *
*    CITM, CIP1TM,     I-TH INTERFACE SPACE GRID POINT FINITE      *
*    CIP2TM            DIFFERENCE EQUATIONS (DIMENSIONLESS).       *
*                                                                 *
*   COTV, C1TV, C2TV - VECTORS OF NON-LINEAR COEFFICIENTS OF THE   *
*                      FIRST SPACE GRID POINT FINITE DIFFERENCE    *
*                      EQUATIONS (DIMENSIONLESS).                  *
*                                                                 *
*     ALPHAV, BETAV, - COEFFICIENTS USED IN CALCULATION OF         *
*     PSI              PRECEDING NON-LINEAR TERMS.                 *
*                                                                 *
*             GRPCNT - SPACE GRID POINT COUNTER.                   *
*                                                                 *
*      MATI1, MATI2, - MATRIX COLUMN POSITION INDICES.             *
*      MATI3                                                       *
*                                                                 *
*******************************************************************

*     DEBUG INIT, SUBTRACE
*     END DEBUG

      SUBROUTINE NLCOEF(VELGRV, VELV, CVTDM, GAMA, DELTA, EPSIV,
     &             CRM2TV, CRM1TV, CBTV, CMM1TM, CMTM, CMP1TM,
     &             CIM2TM, CIM1TM, CITM, CIP1TM, CIP2TM, COTV, C1TV,
     &             C2TV)

      INTEGER NSOL, NGRPTS, NCELLS

      PARAMETER(NSOL = 4, NGRPTS = 2800, NCELLS = 250)

*     ARGUMENTS.

      REAL*4  VELGRV(0:NGRPTS), VELV(0:NGRPTS), CVTDM(3, NCELLS),
     &        GAMA, DELTA, EPSIV(NSOL),
     &        CRM2TV(NSOL), CRM1TV(NSOL), CBTV(NSOL),
     &        CMM1TM(NSOL, NGRPTS-(2*(NCELLS+1))),
     &        CMTM(NSOL, NGRPTS-(2*(NCELLS+1))),
     &        CMP1TM(NSOL, NGRPTS-(2*(NCELLS+1))),
     &        CIM2TM(NSOL, NCELLS-1), CIM1TM(NSOL, NCELLS-1),
     &        CITM(NSOL, NCELLS-1), CIP1TM(NSOL, NCELLS-1),
     &        CIP2TM(NSOL, NCELLS-1), COTV(NSOL), C1TV(NSOL),
     &        C2TV(NSOL)

*     COMMON VARIABLES.

      COMMON /C1/ SOLNUM, IMPNUM, PCONUM, RNUM, RNUMEX, CELNUM,
     &            GRPCEL, COLNUM
      COMMON /C4/ CDIFCM(NSOL, 0:(NGRPTS+2)-NCELLS), SURFL,
     &            AMTRCV(NSOL)
      COMMON /C5/ SPVFO, WVFO, VDIDFO, VCO, VIO, VTO, AIO, ACMO,
     &            ACPO, LCO, RIO, RCO, RBO
      COMMON /C8/ MSTEPV(NCELLS), KSTEP
      COMMON /C11/ AVCDIF, TREF, VREF
      COMMON /C12/ CSIM(NSOL, 0:NGRPTS, 3), CSCM(NSOL, NCELLS, 3),
     &             CELLM(11, NCELLS, 3), MASSM(3, NSOL+1),
     &             STAGEV(NCELLS), CRITCV
      COMMON /C13/ NLEVEL, LVLCNT, GRPTI, CELLI
      COMMON /C16/ GEOMFL

      REAL*4 CDIFCM, AMTRCV, SPVFO, WVFO, VOIDFO, VCO, VIO, VTO,
     &       AIO, ACMO, ACPO, LCO, RIO, RCO, RBO, HSTEPV, KSTEP,
     &       AVCDIF, TREF, VREF, CSIM, CSCM, CELLM, MASSM, CRITCV,
     &       NLEVEL

      INTEGER SOLNUM, IMPNUM, PCONUM, RNUM, RNUMEX, CELNUM, GRPCEL,
     &        COLNUM, SURFL, STAGEV, LVLCNT, GRPTI, CELLI, GEOMFL

*     ROUTINE VARIABLES.

      REAL*4 ALPHAV(NCELLS), BETAV(NCELLS), PSI

      INTEGER GRPCNT, MATI1, MATI2, MATI3

      GAMA = 0.5 * KSTEP

      DO 10 K = 1, CELNUM
         ALPHAV(K) = KSTEP / (2.0 * (HSTEPV(K)**2))
         BETAV(K) = KSTEP / (4.0 * HSTEPV(K))
10    CONTINUE

*     EVALUATE COEFFICIENTS OF FIRST GRID POINT EQUATIONS.

      IF (GEOMFL .EQ. 1) THEN

        DO 20 I = 1, SOLNUM

           C2TV(I) =
     &      (ALPHAV(CELNUM) * (CDIFCM(I, 3) - (CDIFCM(I, 1) / 3.0)))
     &      - (4.0 * BETAV(CELNUM) * VELV(1) / 3.0)

           C1TV(I) = C2TV(I) + (GAMA * (VELGRV(1) + (TREF *
     &               CVTDM(2, CELNUM) / CELLM(2, CELNUM, LVLCNT))))

20      CONTINUE

       ELSE

        DO 25 I = 1, SOLNUM

           C0TV(I) = (ALPHAV(CELNUM) * CDIFCM(I, 1))+
     &               (BETAV(CELNUM) * VELV(1))

           C1TV(I) =
     &       (ALPHAV(CELNUM) * (CDIFCM(I, 3) + CDIFCM(I, 1)))
     &      + (GAMA * (VELGRV(1) + (TREF *
     &       CVTDM(2, CELNUM) / CELLM(2, CELNUM, LVLCNT))))

           C2TV(I) = (ALPHAV(CELNUM) * CDIFCM(I, 3))
     &               - (BETAV(CELNUM) * VELV(1))

25      CONTINUE

      END IF

*     EVALUATE COEFFICIENTS OF THE (R-1)-TH GRID POINT EQUATIONS.

      DELTA = 2.0 * HSTEPV(1) * LCO / AVCDIF

      MATI3 = (((GRPCEL * 2) - 1) * CELNUM) - 1

      DO 30 I = 1, SOLNUM

         IF (SURFL .EQ. 0) THEN

           CRM2TV(I) = (ALPHAV(1) * CDIFCM(I, MATI3-3)) +
     &                 (BETAV(1) * VELV(RNUMEX-1))

           CRM1TV(I) =
     &       (ALPHAV(1) * (CDIFCM(I, MATI3-1) + CDIFCM(I, MATI3-3)))
     &      + (GAMA * (VELGRV(RNUMEX-1) + (TREF * CVTDM(2, 1) /
     &                                      CELLM(2, 1, LVLCNT))))

           CBTV(I) = (ALPHAV(1) * CDIFCM(I, MATI3-1)) -
     &               (BETAV(1) * VELV(RNUMEX-1))

          ELSE

           EPSIV(I) = (3.0 - ((AVCDIF * DELTA * VELV(RNUMEX)) /
     &                (LCO * CDIFCM(I, MATI3))) +
     &                (DELTA * AMTRCV(I) / CDIFCM(I, MATI3)))

           CRM2TV(I)=
     &       (ALPHAV(1) * ((EPSIV(I) * CDIFCM(I, MATI3-3)) -
     &        CDIFCM(I, MATI3-1)) / EPSIV(I)) +
     &       ((EPSIV(I) + 1.0) * BETAV(1) * VELV(RNUMEX-1) /
     &         EPSIV(I))

           CRM1TV(I) =
     &       (ALPHAV(1) * (((EPSIV(I) - 4.0) * CDIFCM(I, MATI3-1))
     &      + (EPSIV(I) * CDIFCM(I, MATI3-3))) / EPSIV(I)) +
     &       (4.0 * BETAV(1) * VELV(RNUMEX-1) / EPSIV(I)) +
     &       (GAMA * (VELGRV(RNUMEX-1) + (TREF * CVTDM(2, 1) /
     &        CELLM(2, 1, LVLCNT))))

           CBTV(I) = ((DELTA * AMTRCV(I)) / (CDIFCM(I, MATI3) *
     &               EPSIV(I))) * ((ALPHAV(1) * CDIFCM(I, MATI3-1))
     &               - (BETAV(1) * VELV(RNUMEX-1)))

         END IF

30    CONTINUE

*     EVALUATE COEFFICIENTS OF M-TH GRID POINT EQUATIONS (INCLUDING
*     INTERFACE POINTS).

      DO 40 I = 1, SOLNUM

         CELLI = CELNUM
         GRPCNT = 3
         GRPTI = 2
         MATI1 = 1
         MATI2 = 1
```

```
            MATI3 = 3
50          CONTINUE
           IF (GRPCNT .LT. GRPCEL) THEN
              CMM1TM(I, MATI1) = (ALPHAV(CELLI) * CDIFCM(I, MATI3))+
     &                           (BETAV(CELLI) * VELV(GRPTI))
              CMTM(I, MATI1) =
     &          (ALPHAV(CELLI) * (CDIFCM(I,MATI3+2) + CDIFCM(I,MATI3)))
     &           + (GAMA * (VELGRV(GRPTI) + (TREF *
     &              CVTDM(2, CELLI) / CELLM(2, CELLI, LVLCNT))))
              CMP1TM(I, MATI1) = (ALPHAV(CELLI) * CDIFCM(I, MATI3+2))
     &                           - (BETAV(CELLI) * VELV(GRPTI))
              GRPCNT = GRPCNT + 1
              GRPTI = GRPTI + 1
              MATI1 = MATI1 + 1
              MATI3 = MATI3 + 2
              IF ((GRPTI .EQ. RNUMEX-1) .OR. (CELNUM .EQ. 1)) GO TO 40
              GO TO 50
            ELSE
              PSI = (CELLM(4, CELLI, LVLCNT) * CDIFCM(I, MATI3+1) *
     &              HSTEPV(CELLI-1)) / (CELLM(4, CELLI-1, LVLCNT) *
     &              CDIFCM(I, MATI3+2) * HSTEPV(CELLI))
              CIM2TM(I, MATI2) = PSI
              CIM1TM(I, MATI2) = 4.0 * PSI
              CITM(I, MATI2) = 3.0 + (3.0 * PSI)
              CIP1TM(I, MATI2) = 4.0
              CIP2TM(I, MATI2) = 1.0
              CELLI = CELLI - 1
              GRPCNT = 2
              GRPTI = GRPTI + 2
              MATI2 = MATI2 + 1
              MATI3 = MATI3 + 3
              GO TO 50
            END IF
40        CONTINUE
          END
*********************************************************************
*                                                                   *
*   ROUTINE NAME   - CSIHAF                                         *
*                                                                   *
*   PURPOSE   - THIS SUBROUTINE IS USED TO ESTIMATE THE REDUCED     *
*               INTERSTITIUM SOLUTE CONCENTRATIONS AT THE N+1/2 TIME *
*               LEVEL.                                              *
*                                                                   *
*   USAGE   - CALL CSIHAF(PDTM, GAMA, DELTA, EPSIV, CRM2TV, CRM1TV, *
*                         CBTV, CMM1TM, CMTM, CMP1TM, CIM2TM,       *
*                         CIM1TM, CITM, CIP1TM, CIP2TM, COTV, C1TV, *
*                         C2TV)                                     *
*                                                                   *
*   ARGUMENTS AND VARIABLES                                         *
*                                                                   *
*                  PDTM - MATRIX OF REDUCED VOLUMETRIC AND SOLUTE   *
*                         PRODUCTION-DEPLETION TERMS.               *
*                                                                   *
*        GAMA, DELTA, - COEFFICIENTS USED IN CALCULATION OF         *
*        EPSIV          INTERSTITIUM CONCENTRATIONS (SEE SUBROUTINE *
*                       NLCOEF FOR DETAILS, DIMENSIONLESS).         *
*                                                                   *
*   CRM2TV, CRM1TV, CBTV, CMM1TM, CMTM, CMP1TM, CIM2TM, CIM1TM,     *
*   CITM, CIP1TM, CIP2TM, COTV, C1TV, C2TV                          *
*                                                                   *
*                       - VECTORS AND MATRICES OF NON-LINEAR        *
*                         COEFFICIENTS OF THE FINITE DIFFERENCE     *
*                         EQUATIONS (SEE SUBROUTINE NLCOEF FOR      *
*                         DETAILS, DIMENSIONLESS).                  *
*                                                                   *
*                GRPCNT - SPACE GRID POINT COUNTER.                 *
*                                                                   *
*         MATI1, MATI2 - MATRIX COLUMN POSITION INDICES.            *
*                                                                   *
*********************************************************************

*         DEBUG INIT, SUBTRACE
*         END DEBUG

          SUBROUTINE CSIHAF(PDTM, GAMA, DELTA, EPSIV, CRM2TV, CRM1TV,
     &                      CBTV, CMM1TM, CMTM, CMP1TM, CIM2TM,
     &                      CIM1TM, CITM, CIP1TM, CIP2TM, COTV, C1TV,
     &                      C2TV)

          INTEGER NSOL, NGRPTS, NCELLS

          PARAMETER(NSOL = 4, NGRPTS = 2500, NCELLS = 250)

*         ARGUMENTS.

          REAL*4  PDTM(NSOL+1, 0:NGRPTS), GAMA, DELTA, EPSIV(NSOL),
     &            CRM2TV(NSOL), CRM1TV(NSOL), CBTV(NSOL),
     &            CMM1TM(NSOL, NGRPTS-(2*(NCELLS+1))),
     &            CMTM(NSOL, NGRPTS-(2*(NCELLS+1))),
     &            CMP1TM(NSOL, NGRPTS-(2*(NCELLS+1))),
     &            CIM2TM(NSOL, NCELLS-1), CIM1TM(NSOL, NCELLS-1),
     &            CITM(NSOL, NCELLS-1), CIP1TM(NSOL, NCELLS-1),
     &            CIP2TM(NSOL, NCELLS-1), COTV(NSOL), C1TV(NSOL),
     &            C2TV(NSOL)

*         COMMON VARIABLES.

          COMMON /C1/ SOLNUM, IMPNUM, PCONUM, RNUM, RNUMEX, CELNUM,
     &              GRPCEL, COLNUM
      COMMON /C3/ CSBOV(NSOL), CSCOV(NSOL), CSIOV(NSOL), CSRV(NSOL)
      COMMON /C4/ CDIFCM(NSOL, 0:(NGRPTS*2)-NCELLS), SURFL,
     &              AMTRCV(NSOL)
      COMMON /C12/ CSIM(NSOL, 0:NGRPTS, 3), CSCM(NSOL, NCELLS, 3),
     &              CELLM(11, NCELLS, 3), MASSM(3, NSOL+1),
     &              STAGEV(NCELLS), CRITCV
      COMMON /C13/ NLEVEL, LVLCNT, GRPTI, CELLI
      COMMON /C16/ GEOMFL

      REAL*4 CSBOV, CSCOV, CSIOV, CSRV, CDIFCM, AMTRCV,
     &       CSIM, CSCM, CELLM, MASSM, CRITCV, NLEVEL

      INTEGER SOLNUM, IMPNUM, PCONUM, RNUM, RNUMEX, CELNUM, GRPCEL,
     &        COLNUM, SURFL, STAGEV, LVLCNT, GRPTI, CELLI, GEOMFL

*     ROUTINE VARIABLES.

      INTEGER GRPCNT, MATI1, MATI2

*     USING INFORMATION AT THE N TIME LEVEL, ESTIMATE CONCENTRATIONS
*     AT THE FIRST AND AT THE (R-1)-TH SPACE GRID POINTS.

      DO 10 I = 1, SOLNUM

         CSIM(I, 1, LVLCNT) =
     &                     ((1.0 - C1TV(I)) * CSIM(I, 1, LVLCNT-1)) +
     &                     (C2TV(I) *  CSIM(I, 2, LVLCNT-1)) -
     &                     (GAMA * PDTM(I+1, 1))

         IF (GEOMFL .EQ. 0) CSIM(I, 1, LVLCNT) =
     &                  CSIM(I, 1, LVLCNT) + (COTV(I) * CSIOV(I))

         CSIM(I, RNUMEX-1, LVLCNT) =
     &            (CRM2TV(I) * CSIM(I, RNUMEX-2, LVLCNT-1)) +
     &            ((1.0 - CRM1TV(I)) * CSIM(I, RNUMEX-1, LVLCNT-1)) +
     &            (CBTV(I) * CSBOV(I)) - (GAMA * PDTM(I+1, RNUMEX-1))

10    CONTINUE

*     ESTIMATE CONCENTRATION AT THE M-TH SPACE GRID POINT (EXCEPT
*     INTERFACE POINTS).

      DO 20 I = 1, SOLNUM

         GRPCNT  = 3
         GRPTI = 2
         MATI1 = 1

30       CONTINUE

         IF (GRPCNT .LT. GRPCEL) THEN

           CSIM(I, GRPTI, LVLCNT) =
     &        (CMM1TM(I, MATI1) * CSIM(I, GRPTI-1, LVLCNT-1)) +
     &        ((1.0 - CMTM(I, MATI1)) * CSIM(I, GRPTI, LVLCNT-1)) +
     &        (CMP1TM(I, MATI1) * CSIM(I, GRPTI+1, LVLCNT-1)) -
     &        (GAMA * PDTM(I+1, GRPTI))

           GRPCNT = GRPCNT + 1
           GRPTI = GRPTI + 1
           MATI1 = MATI1 + 1
           IF (GRPTI .EQ. RNUMEX-1) GO TO 20
           GO TO 30

          ELSE

           GRPCNT = 2
           GRPTI = GRPTI + 2
           GO TO 30

         END IF

20    CONTINUE

*     ESTIMATE CONCENTRATION AT THE ZERO-TH (CENTRE) AND R-TH
*     (SURFACE) SPACE GRID POINTS.

      MATI1 = (((GRPCEL * 2) - 1) * CELNUM) - 1

      DO 40 I = 1, SOLNUM

         IF (GEOMFL .EQ. 1) THEN

           CSIM(I, 0, LVLCNT) = ((4.0 / 3.0) * CSIM(I, 1, LVLCNT)) -
     &                          ((1.0 / 3.0) * CSIM(I, 2, LVLCNT))

          ELSE

           CSIM(I, 0, LVLCNT) = CSIOV(I)

         END IF

         IF (SURFL .EQ. 0) THEN

           CSIM(I, RNUMEX, LVLCNT) = CSBOV(I)

          ELSE

           CSIM(I, RNUMEX, LVLCNT) =
     &       (((DELTA * AMTRCV(I)) / (CDIFCM(I, MATI1) * EPSIV(I))) +
     &        CSBOV(I)) +
     &       ((4.0 / EPSIV(I)) * CSIM(I, RNUMEX-1, LVLCNT)) -
     &       ((1.0 / EPSIV(I)) * CSIM(I, RNUMEX-2, LVLCNT))

         END IF

40    CONTINUE

*     ESTIMATE CONCENTRATION AT THE I-TH INTERFACE POINT.

      IF (CELNUM .EQ. 1) RETURN

      DO 50 I = 1, SOLNUM

         GRPTI = GRPCEL - 1
         MATI2 = 1

50       CONTINUE
```

```
         CSIM(I, GRPTI, LVLCNT) = - (1.0 / CITM(I, MATI2)) *
     &     ((CIP2TM(I, MATI2) * CSIM(I, GRPTI+3, LVLCNT)) -
     &      (CIP1TM(I, MATI2) * CSIM(I, GRPTI+2, LVLCNT)) +
     &      (CIM2TM(I, MATI2) * CSIM(I, GRPTI-2, LVLCNT)) -
     &      (CIM1TM(I, MATI2) * CSIM(I, GRPTI-1, LVLCNT)))

         CSIM(I, GRPTI+1, LVLCNT) = CSIM(I, GRPTI, LVLCNT)

         GRPTI = GRPTI + GRPCEL
         MATI2 = MATI2 + 1
         IF (GRPTI .EQ. RNUMEX) GO TO 50
         GO TO 60

50    CONTINUE

      END

************************************************************************
*                                                                      *
*  ROUTINE NAME  - CELLCH                                              *
*                                                                      *
*  PURPOSE  - THIS SUBROUTINE USES A DIFFERENTIAL EQUATION SOLVER      *
*             (STIFF METHOD OF GEAR) TO EVALUATE, FOR A GIVEN TIME     *
*             INTERVAL (REAL TIME BASIS) AND FOR ALL CELLS             *
*             SIMULTANEOUSLY, THE CHANGES IN THE REDUCED CELLULAR,     *
*             INTERSTITIUM AND TOTAL UNIT CELL VOLUMES, THE            *
*             CHANGES IN THE REDUCED APOPLASTIC, PLASMALEMMAL AND      *
*             PLASMODESMAL TRANSFER AREAS, THE CHANGES IN THE          *
*             REDUCED CELL GEOMETRICAL MENSURATIONS AND FINALLY,       *
*             THE CHANGES IN THE REDUCED CELL SOLUTE                   *
*             CONCENTRATIONS.                                          *
*                                                                      *
*  USAGE  - CALL CELLCH(*M)                                            *
*                                                                      *
*  ARGUMENTS AND VARIABLES                                             *
*                                                                      *
*                *M - ALTERNATE RETURN STATEMENT. M IS THE RETURN      *
*                     LABEL IN THE CALLING PROGRAM. THIS ALTERNATE     *
*                     RETURN IS USED WHEN AN ERROR IS DETECTED IN      *
*                     SUBROUTINE DGEAR.                                *
*                                                                      *
*           NCOUNT - POSITION COUNTER.                                 *
*                                                                      *
*  REMARKS - SUBROUTINE DGEAR IS CALLED TO PERFORM THE INTEGRATION     *
*            OF THE SYSTEM OF DIFFERENTIAL EQUATIONS. SEE ROUTINE      *
*            DESCRIPTION IN IMSL LIBRARY FOR DETAILS ON THE            *
*            ALGORITHM AND ON ITS USAGE. NOTE THAT THE INTEGRATION     *
*            IS PERFORMED ON REAL TIME BASIS.                          *
*                                                                      *
************************************************************************

*     DEBUG INIT, SUBTRACE
*     END DEBUG

      SUBROUTINE CELLCH(*)

      INTEGER NSOL, NGRPTS, NCELLS

      PARAMETER(NSOL = 4, NGRPTS = 2500, NCELLS = 250)

*     COMMON VARIABLES.

      COMMON /C1/ SOLNUM, IMPNUM, PCONUM, RNUM, RNUMEX, CELNUM,
     &            GRPCEL, COLNUM
      COMMON /C8/ GRTOL, GRSTEP, CNTOL, ITEMAX
      COMMON /C9/ HSTEPV(NCELLS), KSTEP
      COMMON /C11/ AVCDIF, TREF, VREF
      COMMON /C12/ CSIM(NSOL, 0:NGRPTS, 3), CSCM(NSOL, NCELLS, 3),
     &             CELLM(11, NCELLS, 3), MASSM(3, NSOL+1),
     &             STAGEV(NCELLS), CRITCV
      COMMON /C13/ NLEVEL, LVLCNT, GRPTI, CELLI

      REAL*4 GRTOL, GRSTEP, CNTOL, HSTEPV, KSTEP, AVCDIF, TREF,
     &       VREF, CSIM, CSCM, CELLM, MASSM, CRITCV, NLEVEL

      INTEGER SOLNUM, IMPNUM, PCONUM, RNUM, RNUMEX, CELNUM, GRPCEL,
     &        COLNUM, ITEMAX, STAGEV, LVLCNT, GRPTI, CELLI

*     ROUTINE VARIABLES.

      REAL*4 X, H, Y(NCELLS*(11+NSOL)), XEND, TOL,
     &       WK(11*(NCELLS*(11+NSOL)))

      INTEGER NEQN, METH, MITER, INDEX, IWK(1), IER, NCOUNT

      EXTERNAL GRFUNC, GRDUM

*     SET UP PARAMETERS REQUIRED BY IMSL ROUTINE DGEAR.

*     NUMBER OF EQUATIONS.

      NEQN = CELNUM * (11 + SOLNUM)

*     INITIAL VALUE OF INDEPENDENT VARIABLE, I.E., CURRENT TIME,
*     IN SEC.

      X = NLEVEL * TREF

*     TIME VALUE AT WHICH SOLUTION IS DESIRED, IN SEC.

      XEND = (NLEVEL + (0.5 * KSTEP)) * TREF

*     INITIAL INTEGRATION STEP TO BE USED BY THE ROUTINE, IN SEC.

      H = ((0.5 * KSTEP) * TREF) * GRSTEP

*     TOLERANCE FOR ERROR CONTROL.

      TOL = GRTOL

*     INDICATORS.

      METH = 2
      MITER = 3
      INDEX = 1

*     INITIALIZE VECTOR OF DEPENDENT VARIABLE VALUES. THE
*     ELEMENTS OF THE Y VECTOR ARE DEFINED AS FOLLOWS (AT ANY TIME):
```

```
*      FROM FIRST CELL TO CELNUM-TH CELL:
*
*      Y(1) TO Y(CELNUM) - REDUCED CELLULAR VOLUME (VC).
*
*      Y(CELNUM+1) TO Y(2*CELNUM) - REDUCED INTERSTITIUM VOLUME (VI).
*
*      Y((2*CELNUM)+1) TO Y(3*CELNUM) - REDUCED TOTAL UNIT CELL
*                                       VOLUME (VT).
*
*      Y((3*CELNUM)+1) TO Y(4*CELNUM) - REDUCED APOPLASTIC TRANSFER
*                                       AREA (AI).
*
*      Y((4*CELNUM)+1) TO Y(5*CELNUM) - REDUCED PLASMALEMMAL TRANSFER
*                                       AREA (ACM).
*
*      Y((5*CELNUM)+1) TO Y(6*CELNUM) - REDUCED PLASMODESMAL
*                                       TRANSFER AREA (ACP).
*
*      Y((6*CELNUM)+1) TO Y(7*CELNUM) - REDUCED UNIT CELL LENGTH (LC)
*
*      Y((7*CELNUM)+1) TO Y(8*CELNUM) - REDUCED INTERSTITIUM CYLINDER
*                                       RADIUS (RI).
*
*      Y((8*CELNUM)+1) TO Y(9*CELNUM) - REDUCED CELLULAR CYLINDER
*                                       RADIUS (RC).
*
*      Y((9*CELNUM)+1) TO Y(10*CELNUM) - REDUCED BUFFER CYLINDER
*                                        RADIUS (RB).
*
*      Y((10*CELNUM)+1) TO Y(11*CELNUM) - INTERSTITIUM RESISTANCE
*                                         FACTOR (IRESF).
*
*      Y((11*CELNUM)+1) TO Y(CELNUM*(11+SOLNUM)
*
*             - REDUCED CELL SOLUTE CONCENTRATIONS ARRANGED FROM
*               FIRST CELL TO CELNUM-TH CELL (SOLNUM SOLUTES PER
*               CELL).

       DO 10 K = 1, CELNUM
          Y(K) = CELLM(1, K, LVLCNT-1)
          Y(CELNUM+K) = CELLM(2, K, LVLCNT-1)
          Y((2*CELNUM)+K) = CELLM(3, K, LVLCNT-1)
          Y((3*CELNUM)+K) = CELLM(4, K, LVLCNT-1)
          Y((4*CELNUM)+K) = CELLM(5, K, LVLCNT-1)
          Y((5*CELNUM)+K) = CELLM(6, K, LVLCNT-1)
          Y((6*CELNUM)+K) = CELLM(7, K, LVLCNT-1)
          Y((7*CELNUM)+K) = CELLM(8, K, LVLCNT-1)
          Y((8*CELNUM)+K) = CELLM(9, K, LVLCNT-1)
          Y((9*CELNUM)+K) = CELLM(10, K, LVLCNT-1)
          Y((10*CELNUM)+K) = CELLM(11, K, LVLCNT-1)
10     CONTINUE

       NCOUNT = (11 * CELNUM) + 1
       DO 20 J = 1, CELNUM
       DO 20 I = 1, SOLNUM
          Y(NCOUNT) = CSCM(I, J, LVLCNT-1)
          NCOUNT = NCOUNT + 1
20     CONTINUE

*      PERFORM INTEGRATION FOR REQUIRED TIME INTERVAL.

       CALL DGEAR(NEQN, GRFUNC, GRDUM, X, H, Y, XEND, TOL, METH,
     &            MITER, INDEX, IWK, WK, IER)

       IF (IER .GT. 128) THEN

         WRITE(2, *) ' **** ERROR IN SUBROUTINE DGEAR ****'
         WRITE(2, *)
         WRITE(2, *) ' IER: ', IER
         RETURN 1

        ELSE

*        UPDATE MATRICES OF CELL MENSURATIONS AND CELL SOLUTE
*        CONCENTRATIONS.

         DO 30 J = 1, CELNUM
            CELLM(1, J, LVLCNT) = Y(J)
            CELLM(2, J, LVLCNT) = Y(CELNUM+J)
            CELLM(3, J, LVLCNT) = Y((2*CELNUM)+J)
            CELLM(4, J, LVLCNT) = Y((3*CELNUM)+J)
            CELLM(5, J, LVLCNT) = Y((4*CELNUM)+J)
            CELLM(6, J, LVLCNT) = Y((5*CELNUM)+J)
            CELLM(7, J, LVLCNT) = Y((6*CELNUM)+J)
            CELLM(8, J, LVLCNT) = Y((7*CELNUM)+J)
            CELLM(9, J, LVLCNT) = Y((8*CELNUM)+J)
            CELLM(10, J, LVLCNT) = Y((9*CELNUM)+J)
            CELLM(11, J, LVLCNT) = Y((10*CELNUM)+J)
30       CONTINUE

         NCOUNT = (11 * CELNUM) + 1
         DO 40 J = 1, CELNUM
         DO 40 I = 1, SOLNUM
            CSCM(I, J, LVLCNT) = Y(NCOUNT)
            NCOUNT = NCOUNT + 1
40       CONTINUE

       END IF

       END

***********************************************************************
*                                                                     *
*  ROUTINE NAME  - MASSCH                                             *
*                                                                     *
*  PURPOSE  - THIS SUBROUTINE EVALUATES, FOR A GIVEN TIME             *
*             INTERVAL, THE CHANGE IN THE OVERALL MASS OF THE         *
*             TISSUE AND THE MASS OF DIFFUSIBLE COMPONENTS LOST OR     *
*             GAINED BY THE TISSUE DURING THE SAME INTERVAL.          *
*                                                                     *
*  USAGE  - CALL MASSCH(VELV, FLUXPM, FLUXAM)                         *
*                                                                     *
*  ARGUMENTS AND VARIABLES.                                           *
*                                                                     *
*             VELV - VECTOR OF REDUCED VOLUME AVERAGE VELOCITIES.     *
*                                                                     *
*           FLUXPM - MATRIX OF MODIFIED PLASMODESMAL FLUXES,          *
*                    IN GMOLE CM-3 SEC-1.                             *
*                                                                    *
*          FLUXAM - MATRIX OF REDUCED APOPLASTIC FLUXES.             *
*                                                                    *
*      SUM1, SUM2 - SUMMATION VARIABLES.                             *
*                                                                    *
*           DMTDT - CURRENT VALUE OF THE TIME VARIATION OF THE       *
*                   OVERALL MASS OF THE TISSUE (DIMENSIONLESS).      *
*                                                                    *
*           DMIDT - CURRENT VALUE OF THE TIME VARIATION OF THE       *
*                   I-TH SOLUTE MASS IN THE TISSUE.                  *
*                                                                    *
*  REMARKS  - EULER'S METHOD IS EMPLOYED TO PERFORM THE              *
*             INTEGRATION WHEN THE INFORMATION IS REQUIRED AT TIME   *
*             LEVEL N + 1/2. AN IMPROVED POLYGON METHOD (MODIFIED    *
*             EULER'S METHOD) IS USED, ON THE OTHER HAND, TO         *
*             ESTIMATE THE CONDITION OF THE TISSUE AT TIME LEVEL     *
*             N + 1.                                                 *
*                                                                    *
**********************************************************************

*     DEBUG INIT, SUBTRACE
*     END DEBUG

      SUBROUTINE MASSCH(VELV, FLUXPM, FLUXAM)

      INTEGER NSOL, NGRPTS, NCELLS

      PARAMETER(NSOL = 4, NGRPTS = 2500, NCELLS = 250)

*     ARGUMENTS.

      REAL*4 VELV(0:NGRPTS), FLUXPM(NSOL+1, NCELLS, 2),
     &       FLUXAM(NSOL+1, 0:NGRPTS, 3)

*     COMMON VARIABLES.

      COMMON /C1/ SOLNUM, IMPNUM, PCONUM, RNUM, RNUMEX, CELNUM,
     &            GRPCEL, COLNUM
      COMMON /C2/ PMVV(NSOL+1), RT
      COMMON /C3/ CSBOV(NSOL), CSCOV(NSOL), CSIOV(NSOL), CSRV(NSOL)
      COMMON /C5/ SPVFO, WVFO, VOIDFO, VCO, VIO, VTO, AIO, ACMO,
     &            ACPO, LCO, RIO, RCO, RBO
      COMMON /C8/ MWV(NSOL+1), TMASSO, MASSOV(NSOL)
      COMMON /C9/ HSTEPV(NCELLS), KSTEP
      COMMON /C11/ AVCDIF, TREF, VREF
      COMMON /C12/ CSIM(NSOL, 0:NGRPTS, 3), CSCM(NSOL, NCELLS, 3),
     &            CELLM(11, NCELLS, 3), MASSM(3, NSOL+1),
     &            STAGEV(NCELLS), CRITCV
      COMMON /C13/ NLEVEL, LVLCNT, GRPTI, CELLI

      REAL*4 PMVV, RT, CSBOV, CSCOV, CSIOV, CSRV, SPVFO,
     &       WVFO, VOIDFO, VCO, VIO, VTO, AIO, ACMO, ACPO, LCO,
     &       RIO, RCO, RBO, MWV, TMASSO, MASSOV, HSTEPV, KSTEP,
     &       AVCDIF, TREF, VREF, CSIM, CSCM, CELLM, MASSM, CRITCV,
     &       NLEVEL

      INTEGER SOLNUM, IMPNUM, PCONUM, RNUM, RNUMEX, CELNUM, GRPCEL,
     &        COLNUM, STAGEV, LVLCNT, GRPTI, CELLI

*     ROUTINE VARIABLES.

      REAL*4 SUM1, SUM2, DMTDT, DMIDT

*     EVALUATE THE CHANGE IN THE OVERALL MASS OF THE TISSUE.

      SUM1 = 0.0
      SUM2 = 0.0

      DO 10 I = 1, SOLNUM
         SUM1 = SUM1 + (FLUXAM(I+1, RNUMEX, 3) * CSRV(I) *
     &      (PMVV(I+1) - (PMVV(1) * MWV(I+1) / MWV(1))))
10    CONTINUE

      DO 20 I = 1, SOLNUM+1
         SUM2 = SUM2 + (FLUXPM(I, 1, 1) * MWV(I))
20    CONTINUE

      DMTDT = REAL(COLNUM) *
     &     ((TREF * VCO * CELLM(6, 1, LVLCNT-1) * SUM2 / TMASSO) -
     &     (((VIO * MWV(1)) / (PMVV(1) * TMASSO)) *
     &     CELLM(4, 1, LVLCNT-1) * (VELV(RNUMEX) - SUM1)))

*     ESTIMATE CONDITION OF TISSUE AT N + 1/2 TIME LEVEL USING
*     EULER'S METHOD, OTHERWISE USE MODIFIED EULER'S METHOD.

      IF (LVLCNT .EQ. 2) THEN
        MASSM(LVLCNT,1) = MASSM(LVLCNT-1,1) + (0.5 * KSTEP * DMTDT)
       ELSE
        MASSM(LVLCNT,1) = MASSM(LVLCNT-2,1) + (KSTEP * DMTDT)
      END IF

*     EVALUATE THE LOSS OR GAIN OF DIFFUSIBLE COMPONENTS BY THE
*     TISSUE.

      DO 30 I = 1, SOLNUM

         DMIDT = REAL(COLNUM) *
     &    ((TREF * VCO * CELLM(8, 1, LVLCNT-1) * FLUXPM(I+1, 1, 1) *
     &    MWV(I+1) / TMASSO) - (VIO * MWV(I+1) * CSRV(I) *
     &    CELLM(4, 1, LVLCNT-1) * FLUXAM(I+1, RNUMEX, 3) / TMASSO))

         IF (LVLCNT .EQ. 2) THEN
           MASSM(LVLCNT, I+1) = MASSM(LVLCNT-1, I+1) +
     &                                     (0.5 * KSTEP * DMIDT)
          ELSE
           MASSM(LVLCNT, I+1) = MASSM(LVLCNT-2, I+1) +
     &                                     (KSTEP * DMIDT)
         END IF

30    CONTINUE

      END

**********************************************************************
*                                                                    *
*  ROUTINE NAME  - CSINP1                                            *
*                                                                    *
*  PURPOSE  - THIS SUBROUTINE IS USED TO ESTIMATE THE REDUCED        *
*              INTERSTITIUM SOLUTE CONCENTRATIONS AT THE N+1 TIME       *
*              LEVEL.                                                   *
*                                                                       *
*  USAGE  - CALL CSINP1(PDTM, GAMA, DELTA, EPSIV, CRM2TV, CRM1TV,       *
*                       CBTV, CMM1TM, CMTM, CMP1TM, CIM2TM,             *
*                       CIM1TM, CITM, CIP1TM, CIP2TM, COTV, C1TV,       *
*                       C2TV)                                           *
*                                                                       *
*  ARGUMENTS AND VARIABLES                                              *
*                                                                       *
*              PDTM - MATRIX OF REDUCED VOLUMETRIC AND SOLUTE           *
*                     PRODUCTION-DEPLETION TERMS.                       *
*                                                                       *
*     GAMA, DELTA, - COEFFICIENTS USED IN CALCULATION OF                *
*     EPSIV          INTERSTITIUM CONCENTRATIONS (SEE SUBROUTINE        *
*                    NLCOEF FOR DETAILS, DIMENSIONLESS).                *
*                                                                       *
*  CRM2TV, CRM1TV, CBTV, CMM1TM, CMTM, CMP1TM, CIM2TM, CIM1TM,          *
*  CITM, CIP1TM, CIP2TM, COTV, C1TV, C2TV                               *
*                                                                       *
*                   - VECTORS AND MATRICES OF NON-LINEAR                *
*                     COEFFICIENTS OF THE FINITE DIFFERENCE             *
*                     EQUATIONS (SEE SUBROUTINE NLCOEF FOR              *
*                     DETAILS, DIMENSIONLESS).                          *
*                                                                       *
*   AV, BV, CV, DV, - VECTORS CONTAINING THE VALUES OF THE              *
*   EV, FV            DIAGONAL ELEMENTS OF THE PENTADIAGONAL            *
*                     MATRIX OF THE SYSTEM OF LINEAR EQUATIONS          *
*                     TO BE SOLVED (SEE SUBROUTINE PENTAG FOR           *
*                     DETAILS, DIMENSIONLESS).                          *
*                                                                       *
*            GRPCNT - SPACE GRID POINT COUNTER.                         *
*                                                                       *
*            GRPTIX - SPACE GRID POINT POSITION INDEX ON THE            *
*                     EXTENDED SPACE GRID.                              *
*                                                                       *
*      MATI1, MATI2 - MATRIX COLUMN POSITION INDICES.                   *
*                                                                       *
*       FIRST, LAST - INDICES OF THE FIRST AND LAST EQUATIONS OF        *
*                     THE SYSTEM TO BE SOLVED.                          *
*                                                                       *
*                VV - SOLUTION VECTOR OF SYSTEM OF LINEAR               *
*                     EQUATIONS.                                        *
*                                                                       *
*************************************************************************

*     DEBUG INIT, SUBTRACE
*     END DEBUG

      SUBROUTINE CSINP1(PDTM, GAMA, DELTA, EPSIV, CRM2TV, CRM1TV,
     &                  CBTV, CMM1TM, CMTM, CMP1TM, CIM2TM,
     &                  CIM1TM, CITM, CIP1TM, CIP2TM, COTV, C1TV,
     &                  C2TV)

      INTEGER NSOL, NGRPTS, NCELLS

      PARAMETER(NSOL = 4, NGRPTS = 2500, NCELLS = 250)

*     ARGUMENTS.

      REAL*4  PDTM(NSOL+1, 0:NGRPTS), GAMA, DELTA, EPSIV(NSOL),
     &        CRM2TV(NSOL), CRM1TV(NSOL), CBTV(NSOL),
     &        CMM1TM(NSOL, NGRPTS-(2*(NCELLS+1))),
     &        CMTM(NSOL, NGRPTS-(2*(NCELLS+1))),
     &        CMP1TM(NSOL, NGRPTS-(2*(NCELLS+1))),
     &        CIM2TM(NSOL, NCELLS-1), CIM1TM(NSOL, NCELLS-1),
     &        CITM(NSOL, NCELLS-1), CIP1TM(NSOL, NCELLS-1),
     &        CIP2TM(NSOL, NCELLS-1), COTV(NSOL), C1TV(NSOL),
     &        C2TV(NSOL)

*     COMMON VARIABLES.

      COMMON /C1/ SOLNUM, IMPNUM, PCONUM, RNUM, RNUMEX, CELNUM,
     &            GRPCEL, COLNUM
      COMMON /C3/ CSBOV(NSOL), CSCOV(NSOL), CSIOV(NSOL), CSRV(NSOL)
      COMMON /C4/ CDIFCM(NSOL, 0:(NGRPTS*2)-NCELLS), SURFL,
     &            AMTRCV(NSOL)
      COMMON /C12/ CSIM(NSOL, 0:NGRPTS, 3), CSCM(NSOL, NCELLS, 3),
     &             CELLM(11, NCELLS, 3), MASSM(3, NSOL+1),
     &             STAGEV(NCELLS), CRITCV
      COMMON /C13/ NLEVEL, LVLCNT, GRPTI, CELLI
      COMMON /C16/ GEOMFL

      REAL*4 CSBOV, CSCOV, CSIOV, CSRV, CDIFCM, AMTRCV,
     &       CSIM, CSCM, CELLM, MASSM, CRITCV, NLEVEL

      INTEGER SOLNUM, IMPNUM, PCONUM, RNUM, RNUMEX, CELNUM, GRPCEL,
     &        COLNUM, SURFL, STAGEV, LVLCNT, GRPTI, CELLI, GEOMFL

*     ROUTINE VARIABLES.

      REAL*4 AV(NGRPTS-NCELLS-1), BV(NGRPTS-NCELLS-1),
     &       CV(NGRPTS-NCELLS-1), DV(NGRPTS-NCELLS-1),
     &       EV(NGRPTS-NCELLS-1), FV(NGRPTS-NCELLS-1),
     &       VV(NGRPTS-NCELLS-1)

      INTEGER GRPCNT, GRPTIX, MATI1, MATI2, FIRST, LAST

*     FOR EACH SOLUTE, USING INFORMATION AT THE N TIME LEVEL AND
*     ESTIMATES OF NON-LINEAR COEFFICIENTS AT THE N+1/2 TIME LEVEL,
*     SET UP VECTORS OF ELEMENTS OF PENTADIAGONAL MATRIX.


      DO 10 I = 1, SOLNUM

*        FIRST SPACE GRID POINT.

         AV(1) = 0.0
         BV(1) = 0.0
         CV(1) = 1.0 + C1TV(I)
         DV(1) = - C2TV(I)
         EV(1) = 0.0
         FV(1) = ((1.0 - C1TV(I)) * CSIM(I, 1, LVLCNT-2)) +
     &           (C2TV(I) *  CSIM(I, 2, LVLCNT-2)) -
     &           (2.0 * GAMA * PDTM(I+1, 1))

         IF (GEOMFL .EQ. 0) FV(1) = FV(1) + (2.0*COTV(I)*CSIOV(I))

*        (N-1)-TH SPACE GRID POINT.
```

```
        AV(RNUM-1) = 0.0
        BV(RNUM-1) = - CRM2TV(I)
        CV(RNUM-1) = 1.0 + CRM1TV(I)
        DV(RNUM-1) = 0.0
        EV(RNUM-1) = 0.0
        FV(RNUM-1) =
     &           (CRM2TV(I) * CSIM(I, RNUMEX-2, LVLCNT-2)) +
     &           ((1.0 - CRM1TV(I)) * CSIM(I, RNUMEX-1, LVLCNT-2)) +
     &           (2.0 * CBTV(I) * CSBOV(I)) -
     &           (2.0 * GAMA * PDTM(I+1, RNUMEX-1))

*       M-TH SPACE GRID POINT (INCLUDING INTERFACE POINTS).

        GRPCNT  = 3
        GRPTIX = 2
        GRPTI = 2
        MATI1 = 1
        MATI2 = 1

20      CONTINUE

        IF (GRPCNT .LT. GRPCEL) THEN

          AV(GRPTI) = 0.0
          BV(GRPTI) = - CMM1TM(I, MATI1)
          CV(GRPTI) = 1.0 + CMTM(I, MATI1)
          DV(GRPTI) = - CMP1TM(I, MATI1)
          EV(GRPTI) = 0.0
          FV(GRPTI) =
     &      (CMM1TM(I, MATI1) * CSIM(I, GRPTIX-1, LVLCNT-2)) +
     &      ((1.0 - CMTM(I, MATI1)) * CSIM(I, GRPTIX, LVLCNT-2)) +
     &      (CMP1TM(I, MATI1) * CSIM(I, GRPTIX+1, LVLCNT-2)) -
     &      (2.0 * GAMA * PDTM(I+1, GRPTIX))

          GRPCNT = GRPCNT + 1
          GRPTIX = GRPTIX + 1
          GRPTI = GRPTI + 1
          MATI1 = MATI1 + 1
          IF ((GRPTIX .EQ. RNUMEX-1) .OR. (CELNUM .EQ. 1)) GO TO 30
          GO TO 20

         ELSE

          AV(GRPTI) = - CIM2TM(I, MATI2)
          BV(GRPTI) = CIM1TM(I, MATI2)
          CV(GRPTI) = - CITM(I, MATI2)
          DV(GRPTI) = CIP1TM(I, MATI2)
          EV(GRPTI) = - CIP2TM(I, MATI2)
          FV(GRPTI) =
     &           (CIM2TM(I, MATI2) * CSIM(I, GRPTIX-2, LVLCNT-2)) -
     &           (CIM1TM(I, MATI2) * CSIM(I, GRPTIX-1, LVLCNT-2)) +
     &           (CITM(I, MATI2) * CSIM(I, GRPTIX, LVLCNT-2)) -
     &           (CIP1TM(I, MATI2) * CSIM(I, GRPTIX+2, LVLCNT-2)) +
     &           (CIP2TM(I, MATI2) * CSIM(I, GRPTIX+3~~, LVLCNT-2))~~

          GRPCNT = 2
          GRPTIX = GRPTIX + 2
          GRPTI = GRPTI + 1
          MATI2 = MATI2 + 1
          GO TO 20

        END IF

30      CONTINUE

*       SOLVE SYSTEM OF LINEAR EQUATIONS.

        FIRST = 1
        LAST = RNUM - 1

        CALL PENTAG(FIRST, LAST, AV, BV, CV, DV, EV, FV, VV)

        GRPCNT = 2
        GRPTIX = 1

        DO 40 K = 1, RNUM-1

            CSIM(I, GRPTIX, LVLCNT) = VV(K)

            IF (GRPCNT .LT. GRPCEL) THEN
              GRPCNT = GRPCNT + 1
              GRPTIX = GRPTIX + 1
             ELSE
              GRPCNT = 2

              CSIM(I, GRPTIX+1, LVLCNT) = CSIM(I, GRPTIX, LVLCNT)

              GRPTIX = GRPTIX + 2
            END IF

40      CONTINUE

*       GET ESTIMATES OF THE INTERSTITIUM CONCENTRATION AT THE
*       CENTRE AND AT THE SURFACE OF THE TISSUE.

        IF (GEOMFL .EQ. 1) THEN

          CSIM(I, 0, LVLCNT) = ((4.0 / 3.0) * CSIM(I, 1, LVLCNT)) -
     &                         ((1.0 / 3.0) * CSIM(I, 2, LVLCNT))

         ELSE

          CSIM(I, 0, LVLCNT) = CSIOV(I)

        END IF

        IF (SURFL .EQ. 0) THEN

          CSIM(I, RNUMEX, LVLCNT) = CSBOV(I)

         ELSE

          MATI1 = (((GRPCEL * 2) - 1) * CELNUM) - 1

          CSIM(I, RNUMEX, LVLCNT) =
     &     (((DELTA * AMTRCV(I)) / (CDIFCM(I, MATI1) * EPSIV(I))) *
     &      CSBOV(I)) +
     &     ((4.0 / EPSIV(I)) * CSIM(I, RNUMEX-1, LVLCNT)) -
     &     ((1.0 / EPSIV(I)) * CSIM(I, RNUMEX-2, LVLCNT))
```

```
            END IF
 10     CONTINUE
        END

*********************************************************************
*                      SECOND LEVEL SUBROUTINES.                    *
*********************************************************************
*                                                                   *
*  ROUTINE NAME  - CYLCEL                                           *
*                                                                   *
*  PURPOSE  - THIS SUBROUTINE EVALUATES THE INITIAL EQUIVALENT     *
*             CYLINDRICAL UNIT CELL MENSURATIONS FROM THE AVERAGE  *
*             TISSUE PROPERTIES.                                    *
*                                                                   *
*  USAGE  - CALL CYLCEL(DIAMCO, GTORT, RPLASM)                      *
*                                                                   *
*  ARGUMENTS AND VARIABLES                                          *
*                                                                   *
*          DIAMCO - INITIAL AVERAGE DIAMETER OF THE CELLS IN THE    *
*                   TISSUE (SPHERE DELIMITED BY PLASMALEMMA),       *
*                   IN CM.                                          *
*                                                                   *
*           GTORT - GEOMETRICAL TORTUOSITY FACTOR (DIMENSIONLESS).  *
*                                                                   *
*          RPLASM - RATIO OF CELL MEMBRANE SURFACE AREA OCCUPIED    *
*                   BY PLASMODESMATA (DIMENSIONLESS).               *
*                                                                   *
*          VOIDFO - INITIAL TISSUE VOID FRACTION, I.E., FRACTION    *
*                   OF THE TOTAL VOLUME OF THE TISSUE WHICH IS      *
*                   EXTRACELLULAR VOLUME (DIMENSIONLESS).           *
*                                                                   *
*              PI - PI CONSTANT (DIMENSIONLESS).                    *
*                                                                   *
*             VCO - INITIAL CELLULAR VOLUME, IN CM3.                *
*                                                                   *
*             VTO - INITIAL TOTAL UNIT CELL VOLUME, IN CM3.         *
*                                                                   *
*             VIO - INITIAL INTERSTITIUM VOLUME, IN CM3.            *
*                                                                   *
*            ACMO - INITIAL CELL MEMBRANE (PLASMALEMMA) SURFACE     *
*                   AREA, IN CM2.                                   *
*                                                                   *
*            ACPO - INITIAL PLASMODESMAL TRANSFER AREA BETWEEN      *
*                   2 NEIGHBOURING CELLS, IN CM2.                   *
*                                                                   *
*             LCO - INITIAL LENGTH OF EQUIVALENT CYLINDRICAL UNIT   *
*                   CELL, IN CM.                                    *
*                                                                   *
*             RIO - INITIAL INTERSTITIUM CYLINDER RADIUS, IN CM.    *
*                                                                   *
*             RCO - INITIAL CELL CYLINDER RADIUS, IN CM.            *
*                                                                   *
*             RBO - INITIAL BUFFER CYLINDER RADIUS, IN CM.          *
*                                                                   *
*             AIO - INITIAL INTERSTITIUM APOPLASTIC TRANSFER AREA,  *
*                   IN CM2.                                         *
*                                                                   *
*********************************************************************

*       DEBUG INIT, SUBTRACE
*       END DEBUG

        SUBROUTINE CYLCEL(DIAMCO, GTORT, RPLASM)

*       ARGUMENTS.

        REAL*4 DIAMCO, GTORT, RPLASM

*       COMMON VARIABLES.

        COMMON /C8/ SPVFO, WVFO, VOIDFO, VCO, VIO, VTO, AIO, ACMO,
     &              ACPO, LCO, RIO, RCO, RBO

        REAL*4 SPVFO, WVFO, VOIDFO, VCO, VIO, VTO, AIO, ACMO, ACPO,
     &         LCO, RIO, RCO, RBO

*       ROUTINE VARIABLES.

        REAL*4 PI

*       INITIAL TISSUE VOID FRACTION.

        VOIDFO = SPVFO + WVFO
        WRITE(2, *) ' VOIDFO: ', VOIDFO

*       INITIAL CELLULAR VOLUME.

        DATA PI/3.14159/
        VCO = (PI * (DIAMCO**3)) / 6.0

*       INITIAL TOTAL UNIT CELL VOLUME.

        VTO = VCO / (1.0 - VOIDFO)

*       INITIAL INTERSTITIUM VOLUME.

        VIO = VTO - VCO
        WRITE(2, *) ' VCO, VIO, VTO: ', VCO, VIO, VTO

*       INITIAL CELL MEMBRANE SURFACE AREA.

        ACMO = (1.0 - RPLASM) * PI * (DIAMCO**2)

*       INITIAL FRACTIONAL PLASMODESMAL TRANSFER AREA.

*       NOTE:  THE TERM "PLASMODESMAL TRANSFER AREA" USED
*              THROUGHOUT THE PROGRAM REFERS TO THE FRACTION OF
*       TOTAL PLASMODESMAL AREA WHICH IS USED, FOR ANY GIVEN CELL,
*       TO DESCRIBE THE SURFACE AVAILABLE FOR SYMPLASTIC TRANSPORT
*       TO/FROM ADJOINING CELLS. THIS FRACTION IS ASSUMED EQUAL FOR
*       ALL 6 POINTS OF CONTACT.

        ACPO = (RPLASM * ACMO) / (6.0 * (1.0 - RPLASM))

*       INITIAL LENGTH OF UNIT CYLINDRICAL CELL.
```

```
      LCO = (8.0 * VTO * (GTORT**(3.0 / 2.0)) / PI)**(1.0 / 3.0)

*     INITIAL RADIUS OF INTERSTITIUM CYLINDER.

      RIO = ACMO / (2.0 * PI * LCO)

*     INITIAL CELL CYLINDER RADIUS.

      RCO = SQRT((VCO / (PI * LCO)) + (RIO**2))

*     INITIAL BUFFER CYLINDER RADIUS.

      RBO = SQRT((RIO**2) - (VIO / (PI * LCO)))
      WRITE(2, *) ' LCO, RIO, RCO, RBO: ', LCO, RIO, RCO, RBO

*     INITIAL INTERSTITIUM APOPLASTIC TRANSFER AREA.

      AIO = PI * ((RIO**2) - (RBO**2))
      WRITE(2, *) ' AIO, ACMO, ACPO: ', AIO, ACMO, ACPO

      END

**********************************************************************
*                                                                    *
*   ROUTINE NAME  - MODCOF                                           *
*                                                                    *
*   PURPOSE   - THIS SUBROUTINE IS USED TO GENERATE THE MODIFIED     *
*               VALUES OF THE EFFECTIVE PLASMALEMMAL AND             *
*               PLASMODESMAL PERMEABILITY COEFFICIENTS.              *
*                                                                    *
*   USAGE   - CALL MODCOF(TEMP, R, PMV, PPV)                         *
*                                                                    *
*   ARGUMENTS AND VARIABLES                                          *
*                                                                    *
*              TEMP - TEMPERATURE AT WHICH PROCESS OCCURS. ASSUMED   *
*                     TO STAY CONSTANT, IN CELSIUS.                  *
*                                                                    *
*                 R - UNIVERSAL GAS CONSTANT, IN DYNE CM GMOLE-1     *
*                     K-1.                                           *
*                                                                    *
*               PMV - VECTOR OF PERMEABILITY COEFFICIENTS (RELATED   *
*                     TO EACH PERMEABLE SPECIES PRESENT IN THE       *
*                     SYSTEM) OF EFFECTIVE CELL MEMBRANE,            *
*                     IN GMOLE DYNE-1 SEC-1.                         *
*                                                                    *
*               PPV - VECTOR OF PERMEABILITY COEFFICIENTS (RELATED   *
*                     TO EACH PERMEABLE SPECIES PRESENT IN THE       *
*                     SYSTEM) OF EQUIVALENT PLASMODESMA MEMBRANE,    *
*                     IN GMOLE DYNE-1 SEC-1.                         *
*                                                                    *
*                RT - PRODUCT OF UNIVERSAL GAS CONSTANT AND          *
*                     TEMPERATURE OF THE SYSTEM, IN DYNE CM GMOLE-1. *
*                                                                    *
*              PHIM - NORMALIZATION FACTOR TO TRANSFORM EFFECTIVE    *
*                     CELL MEMBRANE PERMEABILITY COEFFICIENTS, IN    *
*                     DYNE GMOLE-1.                                  *
*                                                                    *
*              PHIP - NORMALIZATION FACTOR TO TRANSFORM EFFECTIVE    *
*                     PLASMODESMAL PERMEABILITY COEFFICIENTS, IN     *
*                     DYNE GMOLE-1.                                  *
*                                                                    *
*              MPMV - VECTOR OF MODIFIED PMV COEFFICIENTS, IN SEC-1. *
*                                                                    *
*              MPPV - VECTOR OF MODIFIED PPV COEFFICIENTS, IN SEC-1. *
*                                                                    *
**********************************************************************

*     DEBUG INIT, SUBTRACE
*     END DEBUG

      SUBROUTINE MODCOF(TEMP, R, PMV, PPV)

      INTEGER NSOL, NGRPTS, NCELLS

      PARAMETER(NSOL = 4, NGRPTS = 2500, NCELLS = 250)

*     ARGUMENTS.

      REAL*4 TEMP, R, PMV((NSOL+1)*(NSOL+2)/2),
     &       PPV((NSOL+1)*(NSOL+2)/2)

*     COMMON VARIABLES.

      COMMON /C1/ SOLNUM, IMPNUM, PCONUM, RNUM, RNUMEX, CELNUM,
     &            GRPCEL, COLNUM
      COMMON /C2/ PMVV(NSOL+1), RT
      COMMON /C5/ SPVFO, WVFO, VOIDFO, VCO, VIO, VTO, AIO, ACMO,
     &            ACPO, LCO, RIO, RCO, RBO
      COMMON /C10/ MPMV((NSOL+1)*(NSOL+2)/2),
     &             MPPV((NSOL+1)*(NSOL+2)/2)

      REAL*4 PMVV, RT, SPVFO, WVFO, VOIDFO, VCO, VIO, VTO, AIO,
     &       ACMO, ACPO, LCO, RIO, RCO, RBO, MPMV, MPPV

      INTEGER SOLNUM, IMPNUM, PCONUM, RNUM, RNUMEX, CELNUM, GRPCEL,
     &        COLNUM

*     ROUTINE VARIABLES.

      REAL*4 PHIM, PHIP

*     EVALUATE RT CONSTANT.

      RT = R * (TEMP + 273.15)
      WRITE(2, *) ' RT: ', RT

*     EVALUATE PHIM AND PHIP NORMALIZATION FACTORS.

      PHIM = ACMO * RT / VCO
      PHIP = ACPO * RT / VCO
      WRITE(2, *) ' PHIM, PHIP: ', PHIM, PHIP

*     GET MODIFIED VALUES OF PERMEABILITY COEFFICIENTS.

      DO 10 K = 1, PCONUM
         MPMV(K) = PHIM * PMV(K)
         MPPV(K) = PHIP * PPV(K)
         WRITE(2, *) ' MPMV(K), MPPV(K): ', K, MPMV(K), MPPV(K)
10    CONTINUE

      END

****************************************************************
*                                                              *
*  ROUTINE NAME - - REFVAR                                     *
*                                                              *
*  PURPOSE   - THIS SUBROUTINE DEFINES SOME REFERENCE VARIABLES*
*              USED TO TRANSFORM THE ORIGINAL DATA IN REDUCED  *
*              (DIMENSIONLESS) FORM.                           *
*                                                              *
*  USAGE  - CALL REFVAR(IRESFO)                                *
*                                                              *
*  ARGUMENTS AND VARIABLES                                     *
*                                                              *
*           IRESFO - INITIAL RESISTANCE FACTOR OF THE INTERSTITIUM *
*                    COMPLEX (DIMENSIONLESS).                  *
*                                                              *
*             CSRV - VECTOR OF SOLUTE REFERENCE CONCENTRATIONS, IN *
*                    GMOLE CM-3.                               *
*                                                              *
*           PRODCT - PRODUCT VARIABLE USED IN ESTIMATING THE   *
*                    GEOMETRIC MEAN OF THE ABOVE CORRECTED     *
*                    DIFFUSION COEFFICIENTS.                   *
*                                                              *
*           AVCDIF - GEOMETRIC MEAN OF THE PSEUDO-BINARY DIFFUSION *
*                    COEFFICIENTS AT INFINITE DILUTION. USED AS *
*                    REFERENCE DIFFUSION COEFFICIENT, IN CM2 SEC-1. *
*                                                              *
*             TREF - REFERENCE TIME, IN SEC.                   *
*                                                              *
*             VREF - REFERENCE VOLUME AVERAGE VELOCITY, IN CM SEC-1.*
*                                                              *
****************************************************************

*     DEBUG INIT, SUBTRACE
*     END DEBUG

      SUBROUTINE REFVAR(IRESFO)

      INTEGER NSOL, NGRPTS, NCELLS

      PARAMETER(NSOL = 4, NGRPTS = 2500, NCELLS = 250)

*     ARGUMENTS.

      REAL*4 IRESFO

*     COMMON VARIABLES.

      COMMON /C1/ SOLNUM, IMPNUM, PCONUM, RNUM, RNUMEX, CELNUM,
     &            GRPCEL, COLNUM
      COMMON /C3/ CSBOV(NSOL), CSCOV(NSOL), CSIOV(NSOL), CSRV(NSOL)
      COMMON /C5/ SPVFO, WVFO, VOIDFO, VCO, VIO, VTO, AIO, ACMO,
     &            ACPO, LCO, RIO, RCO, RBO
      COMMON /C7/ RNOVL, EMODFL, EMODCO(2), WRESF
      COMMON /C11/ AVCDIF, TREF, VREF
      COMMON /C14/ DIFCV(NSOL), DEGV(NSOL), POLCOM(NSOL, 0:4)

      REAL*4 CSBOV, CSCOV, CSIOV, CSRV, SPVFO, WVFO, VOIDFO,
     &       VCO, VIO, VTO, AIO, ACMO, ACPO, LCO, RIO, RCO, RBO,
     &       RNOVL, EMODCO, WRESF, AVCDIF, TREF, VREF, DIFCV, POLCOM

      INTEGER SOLNUM, IMPNUM, PCONUM, RNUM, RNUMEX, CELNUM, GRPCEL,
     &        COLNUM, EMODFL,DEGV

*     ROUTINE VARIABLES.

      REAL*4 PRODCT

*     DEFINE VECTOR OF SOLUTE REFERENCE CONCENTRATIONS.

      DO 10 I = 1, SOLNUM
         CSRV(I) = (CSCOV(I) + CSBOV(I)) / 2.0
         WRITE(2, *) ' CSRV(I): ', I, CSRV(I)
10    CONTINUE

*     GET INITIAL RESISTANCE FACTOR OF THE INTERSTITIUM COMPLEX.

      IRESFO = (SPVFO + (WVFO * WRESF)) / VOIDFO
      WRITE(2, *) ' IRESFO: ', IRESFO

*     GET GEOMETRIC MEAN OF PSEUDO-BINARY DIFFUSION COEFFICIENTS.
*     DEFINE THE ÆFERENCE DIFFUSION COEFFICIENT.

      PRODCT = 1.0
      DO 20 I = 1, SOLNUM
         PRODCT = PRODCT * DIFCV(I)
20    CONTINUE
      AVCDIF = PRODCT**(1.0 / REAL(SOLNUM))

*     DEFINE REFERENCE TIME.

      TREF = (LCO**2) / AVCDIF

*     DEFINE REFERENCE VOLUME AVERAGE VELOCITY.

      VREF = AVCDIF / LCO
      WRITE(2, *) ' AVCDIF, TREF, VREF: ', AVCDIF, TREF, VREF

      END

****************************************************************
*                                                              *
*  ROUTINE NAME  - CNPAR                                       *
*                                                              *
*  PURPOSE   - THIS SUBROUTINE INITIALIZES SOME PARAMETERS TO BE *
*              USED BY THE CRANK-NICOLSON INTEGRATION ALGORITHM. *
*                                                              *
*  USAGE  - CALL CNPAR(TSAREA, THICKO)                         *
*                                                              *
*  ARGUMENTS AND VARIABLES                                     *
*                                                              *
*          TSAREA - TOTAL SURFACE AREA OF TISSUE EXPOSED TO BATH *
*                   SOLUTION, IN CM2 (SEE SUBROUTINE INIT FOR  *
*              DETAILS).                                              *
*                                                                     *
*        THICKO - INITIAL TISSUE THICKNESS, IN CM.                    *
*                                                                     *
*            PI - PI CONSTANT (DIMENSIONLESS).                        *
*                                                                     *
*        DIAMTO - INITIAL DIAMETER OF THE AVERAGE UNIT CELL,          *
*                 IN CM.                                              *
*                                                                     *
*        TOTCEL - TOTAL NUMBER OF CELLS IN THE STUDIED TISSUE.        *
*                                                                     *
*        CELNUM - NUMBER OF UNITS IN THE LINEAR ARRANGEMENT OF        *
*                 EQUIVALENT CYLINDRICAL CELLS THAT FORMS THE         *
*                 MODEL COLUMN.                                       *
*                                                                     *
*                 THIS VALUE CORRESPONDS TO THE THICKNESS             *
*                 OF THE STUDIED TISSUE DIVIDED BY THE INITIAL        *
*                 AVERAGE DIAMETER OF THE AVERAGE UNIT CELL. IT       *
*                 IS THEREFORE ASSUMED THAT THESE SPHERICAL           *
*                 UNITS HAVE A CUBIC ARRANGEMENT IN THE TISSUE.       *
*                                                                     *
*                 THE THICKNESS USED IN THE CALCULATIONS IS THE       *
*                 CORRECTED THICKNESS TO TAKE INTO ACCOUNT THE        *
*                 CUT CELLS AT THE SURFACE (SEE SUBROUTINE INIT       *
*                 FOR DETAILS).                                       *
*                                                                     *
*        COLNUM - NUMBER OF EQUIVALENT CYLINDRICAL CELL COLUMNS       *
*                 PRESENT IN THE STUDIED TISSUE.                      *
*                                                                     *
*                 THIS VALUE CORRESPONDS TO THE NUMBER OF             *
*                 COLUMNS NECESSARY TO ENSURE THAT THE PRODUCT        *
*                 OF THE UNIT AVERAGE CELL VOLUME BY THE TOTAL        *
*                 NUMBER OF CELLS IS EQUIVALENT TO THE TOTAL          *
*                 VOLUME OF THE STUDIED TISSUE (USING THE             *
*                 CORRECTED THICKNESS).                               *
*                                                                     *
*          RNUM - TOTAL NUMBER OF GRID POINTS IN CRANK-NICOLSON       *
*                 SPACE GRID.                                         *
*                                                                     *
*        RNUMEX - TOTAL NUMBER OF POINTS IN THE EXTENDED SPACE        *
*                 GRID.                                               *
*                                                                     *
***********************************************************************

*     DEBUG INIT, SUBTRACE
*     END DEBUG

      SUBROUTINE CNPAR(TSAREA, THICKO)

*     ARGUMENTS.

      REAL*4 TSAREA, THICKO

*     COMMON VARIABLES.

      COMMON /C1/ SOLNUM, IMPNUM, PCONUM, RNUM, RNUMEX, CELNUM,
     &            GRPCEL, COLNUM
      COMMON /C6/ SPVFO, WVFO, VOIDFO, VCO, VIO, VTO, AIO, ACMO,
     &            ACPO, LCO, RIO, RCO, RBO
      COMMON /C16/ GEOMFL

      REAL*4 SPVFO, WVFO, VOIDFO, VCO, VIO, VTO, AIO, ACMO, ACPO,
     &       LCO, RIO, RCO, RBO

      INTEGER SOLNUM, IMPNUM, PCONUM, RNUM, RNUMEX, CELNUM, GRPCEL,
     &        COLNUM, GEOMFL

*     ROUTINE VARIABLES.

      REAL*4 PI, DIAMTO

      INTEGER TOTCEL

*     GET INITIAL AVERAGE UNIT CELL DIAMETER.

      DATA PI/3.14159/
      DIAMTO = (6.0 * VTO / PI)**(1.0 / 3.0)
      WRITE(2, *) ' DIAMTO: ', DIAMTO

*     EVALUATE TOTAL NUMBER OF CELLS IN TISSUE.

      TOTCEL = INT((TSAREA * THICKO / VTO) + 1.0)
      WRITE(2, *) ' TOTCEL: ', TOTCEL

*     EVALUATE NUMBER OF EQUIVALENT CYLINDRICAL UNIT CELLS IN
*     MODEL COLUMN.

*     CELNUM IS TAKEN AS THE NEAREST INTEGER GREATER THAN
*     THE CALCULATED VALUE (SEE CELNUM DEFINITION).

      IF (GEOMFL .EQ. 1) THICKO = 0.5 * THICKO
      CELNUM = INT((THICKO / DIAMTO) + 1.0)

*     EVALUATE NUMBER OF COLUMNS IN THE STUDIED TISSUE.

      COLNUM = TOTCEL / CELNUM

*     EVALUATE TOTAL NUMBER OF SPACE GRID POINTS (FOR BOTH SPACE
*     GRIDS).

      RNUM = (CELNUM * GRPCEL) - CELNUM
      RNUMEX = (CELNUM * GRPCEL) - 1
      WRITE(2, *) ' CELNUM, COLNUM, RNUM, RNUMEX: ', CELNUM, COLNUM,
     &            RNUM, RNUMEX

      END

***********************************************************************
*                                                                     *
*  ROUTINE NAME  - DCFUNC                                             *
*                                                                     *
*  PURPOSE   - FROM AN EMPIRICAL MODEL (POLYNOMIAL OF DEGREE 3 OR     *
*              LESS) DESCRIBING THE CONCENTRATION DEPENDENCE OF       *
*              THE I-TH SOLUTE PSEUDO-BINARY DIFFUSION COEFFICIENT.   *
*              THIS SUBROUTINE EVALUATES THE LOCAL VALUE OF THE       *
*              COEFFICIENT BASED ON THE LOCAL INTERSTITIUM            *
*              CONCENTRATION OF THE SOLUTE. THEN IT CORRECTS THE      *
*              ESTIMATE TO TAKE INTO ACCOUNT THE RESISTANCE OF THE    *
*              INTERSTITIUM MICROSTRUCTURE.                          *
*                                                                    *
*  USAGE   - CALL DCFUNC(SOLI)                                       *
*                                                                    *
*  ARGUMENTS AND VARIABLES                                           *
*                                                                    *
*            SOLI - SOLUTE INDEX                                     *
*                                                                    *
*          STARTX - GRID POINT POSITION INDEX ON THE EXTENDED        *
*                   SPACE GRID.                                      *
*                                                                    *
*            MATI - CDIFCM MATRIX COLUMN INDEX.                      *
*                                                                    *
*          NCOUNT - POSITION COUNTER FOR ELEMENTS OF VECTOR CSIV.    *
*                                                                    *
*            CSIV - VECTOR OF CONCENTRATION VALUES TO BE USED IN     *
*                   THE EMPIRICAL RELATION, IN GMOLE CM-3.           *
*                                                                    *
*           POLYN - CURRENT CALCULATED VALUE OF THE POLYNOMIAL       *
*                   (DIMENSIONLESS). THE POLYNOMIAL IS DEFINED AS    *
*                   FOLLOWS (FOR ALL SOLUTES):                       *
*                                                                    *
*  LN (D(I) / D(I) INFINITY) = A0 + A1*CS(I) + ... + AN*CS(I)**N     *
*                                                                    *
*           DCVAL - CURRENT VALUE OF THE LOCAL PSEUDO-BINARY         *
*                   DIFFUSION COEFFICIENT.                           *
*                                                                    *
*          CDIFCM - MATRIX OF CORRECTED PSEUDO-BINARY COEFFICIENTS   *
*                   (IN REDUCED FORM).                               *
*                                                                    *
**********************************************************************

*     DEBUG INIT, SUBTRACE
*     END DEBUG

      SUBROUTINE DCFUNC(SOLI)

      INTEGER NSOL, NGRPTS, NCELLS

      PARAMETER(NSOL = 4, NGRPTS = 2500, NCELLS = 250)

*     ARGUMENTS.

      INTEGER SOLI

*     COMMON VARIABLES.

      COMMON /C1/ SOLNUM, IMPNUM, PCONUM, RNUM, RNUMEX, CELNUM,
     &            GRPCEL, COLNUM
      COMMON /C3/ CSBOV(NSOL), CSCOV(NSOL), CSIOV(NSOL), CSRV(NSOL)
      COMMON /C4/ CDIFCM(NSOL, 0:(NGRPTS*2)-NCELLS), SURFL,
     &            AMTRCV(NSOL)
      COMMON /C11/ AVCDIF, TREF, VREF
      COMMON /C12/ CSIM(NSOL, 0:NGRPTS, 3), CSCM(NSOL, NCELLS, 3),
     &             CELLM(11, NCELLS, 3), MASSM(3, NSOL+1),
     &             STAGEV(NCELLS), CRITCV
      COMMON /C13/ NLEVEL, LVLCNT, GRPTI, CELLI
      COMMON /C14/ DIFCV(NSOL), DEGV(NSOL), POLCOM(NSOL, 0:4)

      REAL*4 CSBOV, CSCOV, CSIOV, CSRV, CDIFCM, AMTRCV, AVCDIF,
     &       TREF, VREF, CSIM, CSCM, CELLM, MASSM, CRITCV, NLEVEL,
     &       DIFCV, POLCOM

      INTEGER SOLNUM, IMPNUM, PCONUM, RNUM, RNUMEX, CELNUM, GRPCEL,
     &        COLNUM, SURFL, STAGEV, LVLCNT, GRPTI, CELLI, DEGV

*     ROUTINE VARIABLES.

      REAL*4 CSIV(((NGRPTS/NCELLS)*2)-1), POLYN, DCVAL

      INTEGER STARTX, MATI, NCOUNT

*     SET UP VECTOR OF LOCAL INTERSTITIUM CONCENTRATIONS.

      STARTX = RNUMEX - (CELLI * GRPCEL)
      MATI = ((GRPCEL * 2) - 1) * (CELNUM - CELLI)

      NCOUNT = 1
      K = 1

10    CONTINUE

      CSIV(NCOUNT) = CSIM(SOLI, STARTX+K, LVLCNT) * CSRV(SOLI)

      IF (K .EQ. GRPCEL) GO TO 20

      CSIV(NCOUNT+1) = ((CSIM(SOLI, STARTX+K, LVLCNT) +
     &         CSIM(SOLI, STARTX+K+1, LVLCNT)) / 2.0) * CSRV(SOLI)

      NCOUNT = NCOUNT + 2
      K = K + 1
      GO TO 10

20    CONTINUE

      DO 30 I = 1, NCOUNT

*        USING HORNER'S RULE, COMPUTE THE VALUE OF THE POLYNOMIAL
*        FOR THE GIVEN CONCENTRATION.

         POLYN = 0.0
         DO 40 J = 0, DEGV(SOLI)
            K = DEGV(SOLI) - J
            POLYN = (POLYN * CSIV(I)) + POLCOM(SOLI, K)
40       CONTINUE

*        GET THE LOCAL VALUE OF THE DIFFUSION COEFFICIENT.

         DCVAL = DIFCV(SOLI) * EXP(POLYN)

*        CORRECT THE ABOVE COEFFICIENT AND PUT IT IN REDUCED FORM.
*        UPDATE MATRIX OF REDUCED COEFFICIENTS.

         CDIFCM(SOLI, MATI) = CELLM(11, CELLI, LVLCNT) * DCVAL /
     &                        AVCDIF

         MATI = MATI + 1

30    CONTINUE
```

```
      END

***********************************************************************
*                                                                     *
*  ROUTINE NAME  - FLUXM                                              *
*                                                                     *
*  PURPOSE  - THIS SUBROUTINE EVALUATES THE LOCAL TRANSMEMBRANE       *
*             FLUXES FOR GIVEN EXTRACELLULAR AND INTRACELLULAR        *
*             SOLUTE CONCENTRATIONS AND INTRACELLULAR HYDROSTATIC     *
*             PRESSURE.                                               *
*                                                                     *
*  USAGE  - CALL FLUXM(FLUXMM)                                        *
*                                                                     *
*  ARGUMENTS AND VARIABLES                                            *
*                                                                     *
*          FLUXMM - MATRIX OF MODIFIED TRANSMEMBRANE FLUXES, IN       *
*                   GMOLE CM-3 SEC-1.                                 *
*                                                                     *
*            MLMM - MATRIX OF MODIFIED CELL MEMBRANE                  *
*                   PHENOMENOLOGICAL COEFFICIENTS (LM FORM), IN       *
*                   GMOLE CM-3 SEC-1.                                 *
*                                                                     *
*          FORCEV - VECTOR OF REDUCED THERMODYNAMIC FORCE             *
*                   DIFFERENCE GRADIENTS.                             *
*                                                                     *
***********************************************************************

*     DEBUG INIT, SUBTRACE
*     END DEBUG

      SUBROUTINE FLUXM(FLUXMM)

      INTEGER NSOL, NGRPTS, NCELLS

      PARAMETER(NSOL = 4, NGRPTS = 2500, NCELLS = 250)

*     ARGUMENTS.

      REAL*4 FLUXMM(NSOL+1, 0:NGRPTS)

*     COMMON VARIABLES.

      COMMON /C1/ SOLNUM, IMPNUM, PCONUM, RNUM, RNUMEX, CELNUM,
     &          GRPCEL, COLNUM
      COMMON /C13/ NLEVEL, LVLCNT, GRPTI, CELLI

      REAL*4 NLEVEL

      INTEGER SOLNUM, IMPNUM, PCONUM, RNUM, RNUMEX, CELNUM, GRPCEL,
     &        COLNUM, LVLCNT, GRPTI, CELLI

*     ROUTINE VARIABLES.

      REAL*4 MLMM(NSOL+1, NSOL+1), FORCEV(NSOL+1)

*     GET VALUES OF LOCAL MODIFIED CELL MEMBRANE PHENOMENOLOGICAL
*     COEFFICIENTS. PUT THEM IN A FORM SUITABLE FOR THE CALCULATION
*     OF THE FLUXES, I.E., MATRIX OF LM COEFFICIENTS.

      CALL BUILDM(MLMM)

*     GET ESTIMATES OF DIFFERENCE GRADIENTS OF LOCAL THERMODYNAMIC
*     FORCES ACROSS EFFECTIVE CELL MEMBRANE.

      CALL FGRADM(FORCEV)

*     EVALUATE LOCAL FLUXES.

      DO 10 I = 1, SOLNUM+1
         FLUXMM(I, GRPTI) = 0.0
         DO 10 K = 1, SOLNUM+1
            FLUXMM(I, GRPTI) = FLUXMM(I, GRPTI) + (MLMM(I, K) *
     &                         FORCEV(K))
10    CONTINUE

*     DUE TO THE DEFINITION OF A DIFFERENCE GRADIENT AS:

*        DELTA = QUANTITY INSIDE THE CELL - QUANTITY OUTSIDE THE CELL

*     AN EFFLUX IS POSITIVE WHILE AN INFLUX IS NEGATIVE. TO STAY IN
*     AGREEMENT WITH THE CONVENTION THAT AN EFFLUX IS NEGATIVE
*     (EQUIVALENT TO A LOSS) AND AN INFLUX IS POSITIVE (EQUIVALENT
*     TO A GAIN), THE SIGN OF THE FLUX VALUES MUST THEREFORE BE
*     INVERTED.

      DO 20 I = 1, SOLNUM+1
         FLUXMM(I, GRPTI) = - FLUXMM(I, GRPTI)
20    CONTINUE

      END

***********************************************************************
*                                                                     *
*  ROUTINE NAME  - FLUXP                                              *
*                                                                     *
*  PURPOSE  - THIS SUBROUTINE EVALUATES THE J-TH CELL MODIFIED        *
*             PLASMODESMAL FLUXES (J, (J-1)-TH CELL INTERFACE)        *
*             FOR GIVEN J, (J-1)-TH CELL SOLUTE CONCENTRATIONS        *
*             AND HYDROSTATIC PRESSURES.                              *
*                                                                     *
*  USAGE  - CALL FLUXP(FLUXPM)                                        *
*                                                                     *
*  ARGUMENTS AND VARIABLES                                            *
*                                                                     *
*          FLUXPM - MATRIX OF MODIFIED PLASMODESMAL FLUXES, IN        *
*                   GMOLE CM-3 SEC-1.                                 *
*                                                                     *
*            MLPM - MATRIX OF MODIFIED PLASMODESMAL                   *
*                   PHENOMENOLOGICAL COEFFICIENTS (LP FORM), IN       *
*                   GMOLE CM-3 SEC-1.                                 *
*                                                                     *
*          FORCEV - VECTOR OF REDUCED THERMODYNAMIC FORCE             *
*                   DIFFERENCE GRADIENTS.                             *
*                                                                     *
***********************************************************************
```

```
*     DEBUG INIT, SUBTRACE
*     END DEBUG

      SUBROUTINE FLUXP(FLUXPM)

      INTEGER NSOL, NGRPTS, NCELLS

      PARAMETER(NSOL = 4, NGRPTS = 2500, NCELLS = 250)

*     ARGUMENTS.

      REAL*4 FLUXPM(NSOL+1, NCELLS, 2)

*     COMMON VARIABLES.

      COMMON /C1/ SOLNUM, IMPNUM, PCONUM, RNUM, RNUMEX, CELNUM,
     &            GRPCEL, COLNUM
      COMMON /C13/ NLEVEL, LVLCNT, GRPTI, CELLI

      REAL*4 NLEVEL

      INTEGER SOLNUM, IMPNUM, PCONUM, RNUM, RNUMEX, CELNUM, GRPCEL,
     &        COLNUM, LVLCNT, GRPTI, CELLI

*     ROUTINE VARIABLES.

      REAL*4 MLPM(NSOL+1, NSOL+1), FORCEV(NSOL+1)

      IF ((NLEVEL .LE. 0.0) .AND. (CELLI .EQ. 1)) THEN

        DO 10 I = 1, SOLNUM+1
           FLUXPM(I, CELLI, 1) = 0.0
10      CONTINUE

       ELSE IF (CELLI .EQ. CELNUM+1) THEN

              DO 20 I = 1, SOLNUM+1
                 FLUXPM(I, CELLI-1, 2) = 0.0
20            CONTINUE

             ELSE

*             GET VALUES OF J, (J-1)-TH CELL INTERFACE
*             PLASMODESMAL PHENOMENOLOGICAL COEFFICIENTS.
*             PUT THEM IN A FORM SUITABLE FOR THE CALCULATION
*             OF THE FLUXES, I.E., MATRIX OF LP COEFFICIENTS.

              CALL BUILDP(MLPM)

*             GET ESTIMATES OF THE DIFFERENCE GRADIENTS OF THE
*             THERMODYNAMIC FORCES ACROSS THE INTERFACE.

              CALL FGRADP(FORCEV)

*             EVALUATE PLASMODESMAL FLUXES.

              DO 30 I = 1, SOLNUM+1
                 FLUXPM(I, CELLI, 1) = 0.0
                 DO 30 K = 1, SOLNUM+1
                    FLUXPM(I, CELLI, 1) = FLUXPM(I, CELLI, 1) +
     &                                    (MLPM(I, K) * FORCEV(K))
30            CONTINUE

*             DUE TO THE DEFINITION OF A DIFFERENCE GRADIENT AS:

*              DELTA = QUANTITY INSIDE THE J-TH CELL - QUANTITY
*                      IN THE (J-1)-TH CELL

*             AN EFFLUX IS POSITIVE WHILE AN INFLUX IS NEGATIVE. TO
*             STAY IN AGREEMENT WITH THE CONVENTION THAT AN EFFLUX
*             IS NEGATIVE (EQUIVALENT TO A LOSS) AND AN INFLUX IS
*             POSITIVE (EQUIVALENT TO A GAIN), THE SIGN OF THE FLUX
*             VALUES MUST THEREFORE BE INVERTED.

              DO 40 I = 1, SOLNUM+1
                 FLUXPM(I, CELLI, 1) = - FLUXPM(I, CELLI, 1)
40            CONTINUE

*             THE J, (J+1)-TH CELL INTERFACE FLUXES OF THE
*             PRECEDING CELL IN THE SEQUENCE ((J-1)-TH CELL) ARE
*             SIMPLY THE NEGATIVE VALUES OF THE J, (J-1)-TH CELL
*             INTERFACE FLUXES OF THE CURRENT J-TH CELL.

              IF (CELLI .NE. 1) THEN

                DO 50 I = 1, SOLNUM+1
                   FLUXPM(I, CELLI-1, 2) = - FLUXPM(I, CELLI, 1)
50              CONTINUE

              END IF

      END IF

      END

*****************************************************
*                                                   *
*  ROUTINE NAME  - CVFUNC                           *
*                                                   *
*  PURPOSE  - THIS SUBROUTINE EVALUATES THE CURRENT J-TH CELL
*             REDUCED CELLULAR, INTERSTITIUM AND TOTAL UNIT
*             VOLUME TIME DERIVATIVES.              *
*                                                   *
*  USAGE  - CALL CVFUNC(FLUXMM, FLUXPM, CVTDM)      *
*                                                   *
*  ARGUMENTS AND VARIABLES                          *
*                                                   *
*          FLUXMM - MATRIX OF MODIFIED TRANSMEMBRANE FLUXES, IN
*                   GMOLE CM-3 SEC-1.               *
*                                                   *
*          FLUXPM - MATRIX OF MODIFIED PLASMODESMAL FLUXES,
*                   IN GMOLE CM-3 SEC-1.            *
*                                                   *
*           CVTDM - MATRIX OF UNIT CELL VOLUME TIME DERIVATIVES,
*                   IN SEC-1.                       *
*                                                   *
*          STARTX - GRID POINT POSITION INDEX ON THE EXTENDED
*                   SPACE GRID.                                  *
*                                                                *
*        SUMV, SUM - SUMMATION VARIABLES.                        *
*                                                                *
* X, Y, NX, C, IC - VARIABLES USED IN SUBROUTINES IQHSCU AND     *
* A, B, Q, IER      DCSQDU. SEE IMSL LIBRARY ROUTINE DESCRIPTIONS *
*                   FOR DETAILS.                                 *
*                                                                *
*  SUMPM1, SUMPP1 - SUMMATION VARIABLES.                         *
*                                                                *
*  MINPM1, MINPP1 - VALUES OF PLASMODESMAL TRANSFER AREA TO BE   *
*                   USED IN CALCULATION OF CELLULAR VOLUME TIME  *
*                   DERIVATIVE (DIMENSIONLESS).                  *
*                                                                *
*  REMARKS  - THIS SUBROUTINE USES A QUASI-CUBIC HERMITE         *
*             INTERPOLATION PROCEDURE TO GENERATE SPLINE         *
*             COEFFICIENTS THAT ARE USED, THROUGH A CUBIC SPLINE *
*             QUADRATURE ALGORITHM, TO ESTIMATE THE INTEGRAL OF  *
*             THE LOCAL TRANSMEMBRANE VOLUME FLUXES OVER THE     *
*             LENGTH OF THE CELL (SEE IMSL LIBRARY               *
*             FOR DETAILS ON ROUTINE IQHSCU AND DCSQDU).         *
*                                                                *
******************************************************************

*     DEBUG INIT, SUBTRACE
*     END DEBUG

      SUBROUTINE CVFUNC(FLUXMM, FLUXPM, CVTDM)

      INTEGER NSOL, NGRPTS, NCELLS

      PARAMETER(NSOL = 4, NGRPTS = 2500, NCELLS = 250)

*     ARGUMENTS.

      REAL*4 FLUXMM(NSOL+1, 0:NGRPTS), FLUXPM(NSOL+1, NCELLS, 2),
     &       CVTDM(3, NCELLS)

*     COMMON VARIABLES.

      COMMON /C1/ SOLNUM, IMPNUM, PCONUM, RNUM, RNUMEX, CELNUM,
     &            GRPCEL, COLNUM
      COMMON /C2/ PMVV(NSOL+1), RT
      COMMON /C5/ SPVFO, WVFO, VOIDFO, VCO, VIO, VTO, AIO, ACMO,
     &            ACPO, LCO, RIO, RCO, RBO
      COMMON /C8/ HSTEPV(NCELLS), KSTEP
      COMMON /C12/ CSIM(NSOL, 0:NGRPTS, 3), CSCM(NSOL, NCELLS, 3),
     &             CELLM(11, NCELLS, 3), MASSM(3, NSOL+1),
     &             STAGEV(NCELLS), CRITCV
      COMMON /C13/ NLEVEL, LVLCNT, GRPTI, CELLI
      COMMON /C15/ COMPFV(3)

      REAL*4 PMVV, RT, SPVFO, WVFO, VOIDFO, VCO, VIO, VTO, AIO,
     &       ACMO, ACPO, LCO, RIO, RCO, RBO,
     &       HSTEPV, KSTEP, CSIM, CSCM, CELLM, MASSM,
     &       CRITCV, NLEVEL, COMPFV

      INTEGER SOLNUM, IMPNUM, PCONUM, RNUM, RNUMEX, CELNUM, GRPCEL,
     &        COLNUM, STAGEV, LVLCNT, GRPTI, CELLI

*     ROUTINE VARIABLES.

      REAL*4 SUM, SUMV(NGRPTS/NCELLS), X(NGRPTS/NCELLS),
     &       Y(NGRPTS/NCELLS), C(NGRPTS/NCELLS, 3), A, B, Q, SUMPM1,
     &       SUMPP1, MINPM1, MINPP1

      INTEGER STARTX, NX, IC, IER

*     EVALUATE LOCAL TRANSMEMBRANE VOLUME FLUXES.

      STARTX = RNUMEX - (CELLI * GRPCEL)
      DO 10 K = 1, GRPCEL
         SUM = 0.0
         DO 20 I = 1, SOLNUM+1
            SUM = SUM + (FLUXMM(I, STARTX+K) * PMVV(I))
20       CONTINUE
         SUMV(K) = SUM
10    CONTINUE

*     USING QUASI-HERMITE SPLINE, GENERATE MATRIX OF SPLINE
*     COEFFICIENTS.

      SUM = 0.0
      DO 30 K = CELNUM, CELLI+1, -1
         SUM = SUM + CELLM(7, K, LVLCNT)
30    CONTINUE

      X(1) = SUM
      Y(1) = SUMV(1)
      DO 40 K = 1, GRPCEL-1
         X(K+1) = X(K) + HSTEPV(CELLI)
         Y(K+1) = SUMV(K+1)
40    CONTINUE

      NX = GRPCEL
      IC = NGRPTS / NCELLS

      CALL IQHSCU(X, Y, NX, C, IC, IER)

*     INTEGRATE THE SPLINE OVER THE REQUIRED INTERVAL.

      A = X(1)
      B = X(GRPCEL)

      CALL DCSQDU(X, Y, NX, C, IC, A, B, Q, IER)

*     ESTIMATE THE CELLULAR VOLUME TIME DERIVATIVE.

      SUMPM1 = 0.0
      SUMPP1 = 0.0
      DO 50 I = 1, SOLNUM+1
         SUMPM1 = SUMPM1 + (FLUXPM(I, CELLI, 1) * PMVV(I))
         SUMPP1 = SUMPP1 + (FLUXPM(I, CELLI, 2) * PMVV(I))
50    CONTINUE

      IF (CELLI .EQ. 1) THEN
        MINPM1 = CELLM(8, CELLI, LVLCNT)
       ELSE
        MINPM1 =
     &        MIN(CELLM(8, CELLI, LVLCNT), CELLM(8, CELLI-1, LVLCNT))
      END IF

      IF (CELLI .EQ. CELNUM) THEN
        MINPP1 = CELLM(8, CELLI, LVLCNT)
       ELSE
        MINPP1 =
     &        MIN(CELLM(8, CELLI, LVLCNT), CELLM(8, CELLI+1, LVLCNT))
      END IF

      CVTDM(1, CELLI) = (CELLM(8, CELLI, LVLCNT) * Q) +
     &                  (MINPM1 * SUMPM1) + (MINPP1 * SUMPP1)

*     ESTIMATE INTERSTITIUM AND TOTAL UNIT VOLUME TIME DERIVATIVES.

      CVTDM(2, CELLI) = COMPFV(STAGEV(CELLI))*((1.0-VOIDFO)/VOIDFO)*
     &                  CVTDM(1, CELLI)
      CVTDM(3, CELLI) = (1.0 + COMPFV(STAGEV(CELLI)))*(1.0-VOIDFO)*
     &                  CVTDM(1, CELLI)

      END

****************************************************************
*                                                              *
*  ROUTINE NAME  - VELOCT                                      *
*                                                              *
*  PURPOSE  - THIS SUBROUTINE IS USED TO EVALUATE THE LOCAL     *
*             REDUCED VOLUME AVERAGE VELOCITIES FROM ESTIMATES  *
*             OF THE LOCAL VELOCITY GRADIENTS.                  *
*                                                              *
*  USAGE  - CALL VELOCT(VELGRV, VELV)                          *
*                                                              *
*  ARGUMENTS AND VARIABLES                                     *
*                                                              *
*          VELGRV - VECTOR OF REDUCED VOLUME AVERAGE VELOCITY   *
*                   GRADIENTS.                                  *
*                                                              *
*            VELV - VECTOR OF REDUCED VOLUME AVERAGE VELOCITIES.*
*                                                              *
*          STARTX - GRID POINT POSITION INDEX ON THE EXTENDED   *
*                   SPACE GRID.                                 *
*                                                              *
*             SUM - SUMMATION VARIABLE.                        *
*                                                              *
* X, Y, NX, C, IC - VARIABLES USED IN SUBROUTINES IQHSCU AND   *
* A, B, Q, IER      DCSQDU. SEE IMSL LIBRARY ROUTINE DESCRIPTIONS *
*                   FOR DETAILS.                                *
*                                                              *
*           AREAL - APPROXIMATIVE VALUE OF THE VOLUME AVERAGE   *
*                   VELOCITY AT THE UPPER LIMIT OF INTEGRATION, B. *
*                                                              *
* REMARKS  - THIS SUBROUTINE USES A QUASI-CUBIC HERMITE         *
*            INTERPOLATION PROCEDURE TO GENERATE SPLINE         *
*            COEFFICIENTS THAT ARE USED, THROUGH A CUBIC SPLINE *
*            QUADRATURE ALGORITHM, TO ESTIMATE THE INTEGRAL OF  *
*            THE LOCAL VOLUME AVERAGE VELOCITY GRADIENTS AT EACH *
*            GRID POINT ON A CELL BY CELL BASIS (SEE IMSL LIBRARY *
*            FOR DETAILS ON ROUTINE IQHSCU AND DCSQDU).         *
*                                                              *
****************************************************************

*     DEBUG INIT, SUBTRACE
*     END DEBUG

      SUBROUTINE VELOCT(VELGRV, VELV)

      INTEGER NSOL, NGRPTS, NCELLS

      PARAMETER(NSOL = 4, NGRPTS = 2500, NCELLS = 250)

*     ARGUMENTS.

      REAL*4 VELGRV(0:NGRPTS), VELV(0:NGRPTS)

*     COMMON VARIABLES.

      COMMON /C1/ SOLNUM, IMPNUM, PCONUM, RNUM, RNUMEX, CELNUM,
     &            GRPCEL, COLNUM
      COMMON /C8/ HSTEPV(NCELLS), KSTEP
      COMMON /C12/ CSIM(NSOL, 0:NGRPTS, 3), CSCM(NSOL, NCELLS, 3),
     &             CELLM(11, NCELLS, 3), MASSM(3, NSOL+1),
     &             STAGEV(NCELLS), CRITCV
      COMMON /C13/ NLEVEL, LVLCNT, GRPTI, CELLI

      REAL*4 HSTEPV, KSTEP, CSIM, CSCM, CELLM, MASSM, CRITCV, NLEVEL

      INTEGER SOLNUM, IMPNUM, PCONUM, RNUM, RNUMEX, CELNUM, GRPCEL,
     &        COLNUM, STAGEV, LVLCNT, GRPTI, CELLI

*     ROUTINE VARIABLES.

      REAL*4 SUM, X(NGRPTS/NCELLS), Y(NGRPTS/NCELLS),
     &       C(NGRPTS/NCELLS, 3), AREAL, A, B, Q

      INTEGER STARTX, NX, IC, IER

      DO 10 J = 1, CELNUM

         CELLI = J
         STARTX = RNUMEX - (CELLI * GRPCEL)

*        THE VELOCITY AT EACH EXTENDED SPACE GRID POINT IS EVALUATED
*        USING THE CUBIC SPLINE INTEGRATION ROUTINE.

*        USING QUASI-HERMITE SPLINE, GENERATE MATRIX OF SPLINE
*        COEFFICIENTS (FOR J-TH CELL).

         SUM = 0.0
         DO 20 K = CELNUM, CELLI+1, -1
            SUM = SUM + CELLM(7, K, LVLCNT)
20       CONTINUE

         X(1) = SUM
         Y(1) = VELGRV(STARTX+1)
         DO 30 K = 1, GRPCEL-1
            X(K+1) = X(K) + HSTEPV(CELLI)
            Y(K+1) = VELGRV(STARTX+1+K)
30       CONTINUE
```

```
         NX = GRPCEL
         IC = NGRPTS / NCELLS

         CALL IOHSCU(X, Y, NX, C, IC, IER)

*        INTEGRATE THE SPLINE OVER THE REQUIRED INTERVAL

         AREAL = VELV(STARTX+GRPCEL)
         B = X(GRPCEL)

         DO 40 K = 1, GRPCEL-1
            A = X(K)
            CALL DCSQDU(X, Y, NX, C, IC, A, B, Q, IER)
            VELV(STARTX+K) = AREAL - Q
40       CONTINUE

         IF (CELLI .EQ. CELNUM) RETURN
         VELV(STARTX) =
     &    (CELLM(4, CELLI, LVLCNT) / CELLM(4, CELLI+1, LVLCNT)) *
     &    VELV(STARTX+1)

10    CONTINUE

      END

****************************************************************
*                                                              *
*  ROUTINE NAME  - FLUXA                                       *
*                                                              *
*  PURPOSE  - THIS SUBROUTINE EVALUATES THE LOCAL VALUES OF THE *
*             REDUCED APOPLASTIC FLUXES. IT ESTIMATES THE      *
*             RELATIVE CONTRIBUTION OF DIFFUSION AND CONVECTION *
*             IN THE OVERALL MASS TRANSPORT PHENOMENA.         *
*                                                              *
*  USAGE  - CALL FLUXA(VELV, FLUXAM)                           *
*                                                              *
*  ARGUMENTS AND VARIABLES.                                    *
*                                                              *
*            VELV - VECTOR OF REDUCED VOLUME AVERAGE VELOCITIES. *
*                                                              *
*          FLUXAM - MATRIX OF LOCAL VALUES OF REDUCED APOPLASTIC *
*                   FLUXES.                                    *
*                                                              *
*          STARTX - GRID POINT POSITION INDEX ON THE EXTENDED  *
*                   SPACE GRID.                                *
*                                                              *
*            MATI - MATRIX COLUMN INDEX.                       *
*                                                              *
*             SUM - SUMMATION VARIABLE.                        *
*                                                              *
* X, Y, NX, C,    - VARIABLES USED IN SUBROUTINES IOHSCU AND   *
* IC, U, DS, M1,    DCSEVU. SEE IMSL LIBRARY ROUTINE DESCRIPTIONS *
* DDS, M2, IER      FOR DETAILS.                               *
*                                                              *
*  REMARKS  - THIS SUBROUTINE USES A QUASI-CUBIC HERMITE       *
*             INTERPOLATION PROCEDURE TO GENERATE SPLINE       *
*             COEFFICIENTS THAT ARE USED, THROUGH A CUBIC SPLINE *
*             DERIVATIVE EVALUATOR, TO ESTIMATE THE SOLUTE     *
*             CONCENTRATION GRADIENTS AT EACH GRID POINT IN THE *
*             INTERSTITIUM (SEE IMSL LIBRARY FOR DETAILS ON    *
*             ROUTINE IOHSCU AND DCSEVU).                      *
*                                                              *
****************************************************************

*     DEBUG INIT, SUBTRACE
*     END DEBUG

      SUBROUTINE FLUXA(VELV, FLUXAM)

      INTEGER NSOL, NGRPTS, NCELLS

      PARAMETER(NSOL = 4, NGRPTS = 2500, NCELLS = 250)

*     ARGUMENTS.

      REAL*4 VELV(0:NGRPTS), FLUXAM(NSOL+1, 0:NGRPTS, 3)

*     COMMON VARIABLES.

      COMMON /C1/ SOLNUM, IMPNUM, PCONUM, RNUM, RNUMEX, CELNUM,
     &            GRPCEL, COLNUM
      COMMON /C2/ PMVV(NSOL+1), RT
      COMMON /C4/ CDIFCM(NSOL, 0:(NGRPTS*2)-NCELLS), SURFL,
     &            AMTRCV(NSOL)
      COMMON /C8/ HSTEPV(NCELLS), KSTEP
      COMMON /C12/ CSIM(NSOL, 0:NGRPTS, 3), CSCM(NSOL, NCELLS, 3),
     &             CELLM(11, NCELLS, 3), MASSM(3, NSOL+1),
     &             STAGEV(NCELLS), CRITCV
      COMMON /C13/ NLEVEL, LVLCNT, GRPTI, CELLI

      REAL*4 PMVV, RT, CDIFCM, AMTRCV, HSTEPV, KSTEP, CSIM, CSCM,
     &       CELLM, MASSM, CRITCV, NLEVEL

      INTEGER SOLNUM, IMPNUM, PCONUM, RNUM, RNUMEX, CELNUM, GRPCEL,
     &        COLNUM, SURFL, STAGEV, LVLCNT, GRPTI, CELLI

*     ROUTINE VARIABLES.

      REAL*4 SUM, X(NGRPTS/NCELLS), U(NGRPTS/NCELLS),
     &       Y(NGRPTS/NCELLS), C(NGRPTS/NCELLS, 3),
     &       DS(NGRPTS/NCELLS), DDS(1)

      INTEGER STARTX, MATI, NX, IC, IER, M1, M2

      DO 10 I = 1, SOLNUM
      DO 10 J = 1, CELNUM

         CELLI = J
         STARTX = RNUMEX - (CELLI * GRPCEL)
         MATI = ((GRPCEL * 2) - 1) * (CELNUM - CELLI)

*        THE INTERSTITIUM CONCENTRATION GRADIENTS FOR THE I-TH
*        SOLUTE AND THE J-TH CELL ARE EVALUATED USING CUBIC SPLINE
*        DERIVATIVE EVALUATOR.

*        USING QUASI-HERMITE SPLINE, GENERATE MATRIX OF SPLINE
*        COEFFICIENTS (FOR J-TH CELL).
```

```
         SUM = 0.0
         DO 20 K = CELNUM, CELLI+1, -1
            SUM = SUM + CELLM(7, K, LVLCNT)
20       CONTINUE

         X(1) = SUM
         U(1) = X(1)
         Y(1) = CSIM(I, STARTX+1, LVLCNT)
         DO 30 K = 1, GRPCEL-1
            X(K+1) = X(K) + HSTEPV(CELLI)
            U(K+1) = X(K+1)
            Y(K+1) = CSIM(I, STARTX+1+K, LVLCNT)
30       CONTINUE

         NX = GRPCEL
         IC = NGRPTS / NCELLS

         CALL IOHSCU(X, Y, NX, C, IC, IER)

*        EVALUATE LOCAL CONCENTRATION GRADIENTS.

         M1 = GRPCEL
         M2 = 0

         CALL DCSEVU(X, Y, NX, C, IC, U, DS, M1, DDS, M2, IER)

*        EVALUTE LOCAL APOPLASTIC FLUXES.

         DO 40 K = 1, GRPCEL

*           DIFFUSIVE FLUX.

            FLUXAM(I+1, STARTX+K, 1) = - (CDIFCM(I, MATI) * DS(K))

*           CONVECTIVE FLUX.

            FLUXAM(I+1, STARTX+K, 2) = CSIM(I, STARTX+K, LVLCNT) *
     &                                 VELV(STARTX+K)

*           NET FLUX.

            FLUXAM(I+1, STARTX+K, 3) = FLUXAM(I+1, STARTX+K, 1) +
     &                                 FLUXAM(I+1, STARTX+K, 2)

            MATI = MATI + 2

40       CONTINUE

10    CONTINUE

*     ESTIMATE SOLVENT NET FLUX AT EACH EXTENDED SPACE GRID POINT.

      DO 50 J = 0, RNUMEX

         SUM = 0.0
         DO 60 I = 1, SOLNUM
            SUM = SUM + (FLUXAM(I+1, J, 3) * PMVV(I+1))
60       CONTINUE

         FLUXAM(1, J, 3) = (VELV(J) - SUM) / PMVV(1)

50    CONTINUE

      END

**********************************************************************
*                                                                    *
*  ROUTINE NAME  - GRFUNC                                            *
*                                                                    *
*  PURPOSE  - THIS SUBROUTINE IS CALLED BY DGEAR TO PERFORM THE     *
*             SIMULTANEOUS EVALUATION OF THE TIME DERIVATIVES.       *
*             ON INPUT IT RECEIVES THE VALUES NEEDED TO EVALUATE     *
*             THESE RELATIONS, ON OUTPUT IT GIVES THE ESTIMATES      *
*             OF THE DERIVATIVES.                                    *
*                                                                    *
*  USAGE  - CALL GRFUNC(N, X, Y, YPRIME)                             *
*                                                                    *
*  ARGUMENTS AND VARIABLES                                           *
*                                                                    *
*               N - NUMBER OF EQUATIONS.                             *
*                                                                    *
*               X - CURRENT VALUE OF THE INDEPENDENT VARIABLE,       *
*                      TIME, IN SEC.                                 *
*                                                                    *
*               Y - VECTOR OF CURRENT VALUES OF THE DEPENDENT        *
*                      VARIABLES (DIMENSIONLESS).                    *
*                                                                    *
*          YPRIME - VECTOR OF CURRENT VALUES OF THE FIRST            *
*                      DERIVATIVES OF Y WITH RESPECT TO X, IN SEC-1. *
*                                                                    *
*          NCOUNT - POSITION COUNTER.                                *
*                                                                    *
*          GRPCNT - GRID POINT COUNTER.                              *
*                                                                    *
*          FLUXMM - MATRIX OF MODIFIED TRANSMEMBRANE FLUXES, IN      *
*                      GMOLE CM-3 SEC-1.                             *
*                                                                    *
*          FLUXPM - MATRIX OF MODIFIED PLASMODESMAL FLUXES, IN       *
*                      GMOLE CM-3 SEC-1.                             *
*                                                                    *
*           CVTDM - MATRIX OF UNIT CELL VOLUME TIME DERIVATIVES,     *
*                      IN SEC-1.                                     *
*                                                                    *
*           TATDM - MATRIX OF UNIT CELL TRANSFER AREA TIME           *
*                      DERIVATIVES, IN SEC-1.                        *
*                                                                    *
*           GPTDM - MATRIX OF UNIT CELL GEOMETRICAL PARAMETER        *
*                      TIME DERIVATIVES, IN SEC-1.                   *
*                                                                    *
*           CSTDM - MATRIX OF CELL SOLUTE CONCENTRATION TIME         *
*                      DERIVATIVES, IN SEC-1.                        *
*                                                                    *
**********************************************************************

*     DEBUG INIT, SUBTRACE
*     END DEBUG
```

```
      SUBROUTINE GRFUNC(N, X, Y, YPRIME)

      INTEGER NSOL, NGRPTS, NCELLS

      PARAMETER(NSOL = 4, NGRPTS = 2500, NCELLS = 250)

*     ARGUMENTS.

      INTEGER N

      REAL*4 X, Y(N), YPRIME(N)

*     COMMON VARIABLES.

      COMMON /C1/ SOLNUM, IMPNUM, PCONUM, RNUM, RNUMEX, CELNUM,
     &            GRPCEL, COLNUM
      COMMON /C11/ AVCDIF, TREF, VREF
      COMMON /C12/ CSIM(NSOL, 0:NGRPTS, 3), CSCM(NSOL, NCELLS, 3),
     &             CELLM(11, NCELLS, 3), MASSM(3, NSOL+1),
     &             STAGEV(NCELLS), CRITCV
      COMMON /C13/ NLEVEL, LVLCNT, GRPTI, CELLI

      REAL*4 AVCDIF, TREF, VREF, CSIM, CSCM, CELLM, MASSM, CRITCV,
     &       NLEVEL

      INTEGER SOLNUM, IMPNUM, PCONUM, RNUM, RNUMEX, CELNUM, GRPCEL,
     &        COLNUM, STAGEV, LVLCNT, GRPTI, CELLI

*     ROUTINE VARIABLES.

      REAL*4 FLUXMM(NSOL+1, 0:NGRPTS), FLUXPM(NSOL+1, NCELLS, 2),
     &       CVTDM(3, NCELLS), TATDM(3, NCELLS), GPTDM(5, NCELLS),
     &       CSTDM(NSOL, NCELLS)

      INTEGER NCOUNT, GRPCNT

*     UPDATE CURRENT TIME LEVEL.

      NLEVEL = X / TREF

*     TRANSFER Y VECTOR INFORMATION TO CELLM AND CSCM MATRICES.

      DO 10 J = 1, CELNUM

         CELLM(1, J, LVLCNT) = Y(J)

*        IF J-TH CELL HAS REACHED THE THIRD STAGE OF DEHYDRATION,
*        SET STAGE FLAG TO 3.

         IF (CELLM(1, J, LVLCNT) .LE. CRITCV) THEN
           STAGEV(J) = 3
          ELSE IF (STAGEV(J) .NE. 1) THEN
           STAGEV(J) = 2
         END IF

         CELLM(2, J, LVLCNT) = Y(CELNUM+J)
         CELLM(3, J, LVLCNT) = Y((2*CELNUM)+J)
         CELLM(4, J, LVLCNT) = Y((3*CELNUM)+J)
         CELLM(5, J, LVLCNT) = Y((4*CELNUM)+J)
         CELLM(6, J, LVLCNT) = Y((5*CELNUM)+J)
         CELLM(7, J, LVLCNT) = Y((6*CELNUM)+J)
         CELLM(8, J, LVLCNT) = Y((7*CELNUM)+J)
         CELLM(9, J, LVLCNT) = Y((8*CELNUM)+J)
         CELLM(10, J, LVLCNT) = Y((9*CELNUM)+J)
         CELLM(11, J, LVLCNT) = Y((10*CELNUM)+J)
10    CONTINUE

      NCOUNT = (11 * CELNUM) + 1
      DO 20 J = 1, CELNUM
      DO 20 I = 1, SOLNUM
         CSCM(I, J, LVLCNT) = Y(NCOUNT)
         NCOUNT = NCOUNT + 1
20    CONTINUE

*     EVALUATE LOCAL TRANSMEMBRANE FLUXES (FOR ALL SPECIES AND ALL
*     EXTENDED SPACE GRID POINTS, ON A CELL BY CELL BASIS).

      CELLI = 1
      GRPCNT = 1
      DO 30 J = RNUMEX, 0, -1
         GRPTI = J
         CALL FLUXM(FLUXMM)
         IF (GRPCNT .GE. GRPCEL) THEN
           CELLI = CELLI + 1
           GRPCNT = 1
          ELSE
           GRPCNT = GRPCNT + 1
         END IF
30    CONTINUE

*     EVALUATE, FOR EACH CELL, THE PLASMODESMAL FLUXES.

      DO 40 J = 1, CELNUM+1
         CELLI = J
         CALL FLUXP(FLUXPM)
40    CONTINUE

*     EVALUATE, FOR EACH CELL, THE CURRENT CELLULAR, INTERSTITIUM
*     AND TOTAL CELL VOLUME TIME DERIVATIVES.

      DO 50 J = 1, CELNUM
         CELLI = J
         CALL CVFUNC(FLUXMM, FLUXPM, CVTDM)
50    CONTINUE

*     EVALUATE, FOR EACH CELL, THE CURRENT TIME DERIVATIVES OF THE
*     PLASMALEMMAL AND PLASMODESMAL TRANSFER AREAS.

      DO 60 J = 1, CELNUM
         CELLI = J
         CALL TAFUNC(CVTDM, TATDM)
60    CONTINUE

*     EVALUATE, FOR EACH CELL, THE CURRENT TIME DERIVATIVES OF THE
*     UNIT CELL GEOMETRICAL PARAMETERS.

      DO 70 J = 1, CELNUM
         CELLI = J
         CALL GPFUNC(CVTDM, TATDM, GPTDM)
70    CONTINUE
```

```
*     TRANSFER INFORMATION TO YPRIME VECTOR.

      DO 80 K = 1, CELNUM
         YPRIME(K) = CVTDM(1, K)
         YPRIME(CELNUM+K) = CVTDM(2, K)
         YPRIME((2*CELNUM)+K) = CVTDM(3, K)
         YPRIME((3*CELNUM)+K) = TATDM(1, K)
         YPRIME((4*CELNUM)+K) = TATDM(2, K)
         YPRIME((5*CELNUM)+K) = TATDM(3, K)
         YPRIME((6*CELNUM)+K) = GPTDM(1, K)
         YPRIME((7*CELNUM)+K) = GPTDM(2, K)
         YPRIME((8*CELNUM)+K) = GPTDM(3, K)
         YPRIME((9*CELNUM)+K) = GPTDM(4, K)
         YPRIME((10*CELNUM)+K) = GPTDM(5, K)
80    CONTINUE

*     EVALUATE THE CELL SOLUTE CONCENTRATION TIME DERIVATIVES (FOR
*     ALL SOLUTES AND ALL CELLS).

      NCOUNT = (11 * CELNUM) + 1
      DO 90 J = 1, CELNUM
         CELLI = J
         CALL CCFUNC(FLUXMM, FLUXPM, CVTDM, CSTDM)
         DO 90 I = 1, SOLNUM
            YPRIME(NCOUNT) = CSTDM(I, J)
            NCOUNT = NCOUNT + 1
90    CONTINUE

      END

********************************************************************
*                                                                  *
*  ROUTINE NAME  - GRDUM                                           *
*                                                                  *
*  PURPOSE    - DUMMY ROUTINE CALLED BY DGEAR (MITER = 3). SEE     *
*               ROUTINE DESCRIPTION FOR DETAILS.                   *
*                                                                  *
*  USAGE  - CALL GRDUM(N, X, Y, PD)                                *
*                                                                  *
********************************************************************

      SUBROUTINE GRDUM(N, X, Y, PD)

*     ARGUMENTS.

      INTEGER N

      REAL*4 X, Y(N), PD(N, N)

      END

********************************************************************
*                                                                  *
*  ROUTINE NAME  - PENTAC                                          *
*                                                                  *
*  PURPOSE   - THIS SUBROUTINE IS USED TO SOLVE A SYSTEM OF LINEAR *
*              SIMULTANEOUS EQUATIONS ( A X = B ) HAVING A         *
*              PENTADIAGONAL COEFFICIENT BAND MATRIX ( A ).        *
*                                                                  *
*  USAGE  - CALL PENTAC(FIRST, LAST, AV, BV, CV, DV, EV, FV, VV)   *
*                                                                  *
*  ARGUMENTS AND VARIABLES                                         *
*                                                                  *
*           THE EQUATIONS ARE NUMBERED FROM  FIRST  TO  LAST       *
*           AND THEIR SECOND, FIRST LOWER DIAGONAL, MAIN DIAGONAL,*
*           FIRST AND SECOND UPPER DIAGONAL COEFFICIENTS ARE       *
*           STORED IN THE ARRAYS AV, BV, CV, DV, EV RESPECTIVELY.  *
*           FV IS AN ARRAY CONTAINING THE INFORMATION VECTOR B.    *
*           THE COMPUTED SOLUTION VECTOR X (X(FIRST), ...,         *
*           X(LAST)) IS STORED IN THE ARRAY VV.                    *
*                                                                  *
*    DELTAV, LAMDAV, GAMAV - INTERNAL ARRAYS USED IN THE ALGORITHM.*
*                                                                  *
*                 MU, BETA - INTERMEDIATE VARIABLES APPEARING IN   *
*                            THE CALCULATIONS.                     *
*                                                                  *
*  REMARKS   - TO KEEP ROUNDING ERRORS TO A MINIMUM, THE           *
*              CALCULATIONS ARE PERFORMED IN DOUBLE-PRECISION.     *
*              NOTE THAT THE VECTORS THAT ARE INPUT AND OUTPUT ARE *
*              IN SINGLE-PRECISION.                                *
*                                                                  *
********************************************************************

*     DEBUG INIT, SUBTRACE
*     END DEBUG

      SUBROUTINE PENTAC(FIRST, LAST, SAV, SBV, SCV, SDV, SEV,
     &                 SFV, SVV)

      INTEGER NSOL, NGRPTS, NCELLS

      PARAMETER(NSOL = 4, NGRPTS = 2500, NCELLS = 250)

*     ARGUMENTS.

      REAL*4 SAV(NGRPTS-NCELLS-1), SBV(NGRPTS-NCELLS-1),
     &       SCV(NGRPTS-NCELLS-1), SDV(NGRPTS-NCELLS-1),
     &       SEV(NGRPTS-NCELLS-1), SFV(NGRPTS-NCELLS-1),
     &       SVV(NGRPTS-NCELLS-1)

      INTEGER FIRST, LAST

*     ROUTINE VARIABLES.

      REAL*8 AV(NGRPTS-NCELLS-1), BV(NGRPTS-NCELLS-1),
     &       CV(NGRPTS-NCELLS-1), DV(NGRPTS-NCELLS-1),
     &       EV(NGRPTS-NCELLS-1), FV(NGRPTS-NCELLS-1),
     &       VV(NGRPTS-NCELLS-1), DELTAV(NGRPTS-NCELLS-1),
     &       LAMDAV(NGRPTS-NCELLS-1), GAMAV(NGRPTS-NCELLS-1),
     &       MU, BETA

*     CONVERT INCOMING VECTORS IN DOUBLE-PRECISION.

      DO 10 K = FIRST, LAST
         AV(K) = DBLE(SAV(K))
```

```
          BV(K) = DBLE(SBV(K))
          CV(K) = DBLE(SCV(K))
          DV(K) = DBLE(SDV(K))
          EV(K) = DBLE(SEV(K))
          FV(K) = DBLE(SFV(K))
10     CONTINUE

       DELTAV(FIRST) = DV(FIRST) / CV(FIRST)
       LAMDAV(FIRST) = EV(FIRST) / CV(FIRST)
       GAMAV(FIRST) = FV(FIRST) / CV(FIRST)

       MU = CV(FIRST+1) - (BV(FIRST+1) * DELTAV(FIRST))
       DELTAV(FIRST+1) = (DV(FIRST+1) - (BV(FIRST+1) *
     &                   LAMDAV(FIRST))) / MU
       LAMDAV(FIRST+1) = EV(FIRST+1) / MU
       GAMAV(FIRST+1) = (FV(FIRST+1) - (BV(FIRST+1) *
     &                  GAMAV(FIRST))) / MU

       DO 20 I = FIRST+2, LAST-2
          BETA = BV(I) - (AV(I) * DELTAV(I-2))
          MU = CV(I) - (BETA * DELTAV(I-1)) - (AV(I) * LAMDAV(I-2))
          DELTAV(I) = (DV(I) - (BETA * LAMDAV(I-1))) / MU
          LAMDAV(I) = EV(I) / MU
          GAMAV(I) = (FV(I) - (BETA * GAMAV(I-1)) -(AV(I) *
     &              GAMAV(I-2))) / MU
20     CONTINUE

       BETA = BV(LAST-1) - (AV(LAST-1) * DELTAV(LAST-3))
       MU = CV(LAST-1) - (BETA * DELTAV(LAST-2)) - (AV(LAST-1) *
     &     LAMDAV(LAST-3))
       DELTAV(LAST-1) = (DV(LAST-1) - (BETA * LAMDAV(LAST-2))) / MU
       GAMAV(LAST-1) = (FV(LAST-1) - (BETA * GAMAV(LAST-2)) -
     &                 (AV(LAST-1) * GAMAV(LAST-3))) / MU

       BETA = BV(LAST) - (AV(LAST) * DELTAV(LAST-2))
       MU = CV(LAST) - (BETA * DELTAV(LAST-1)) - (AV(LAST) *
     &     LAMDAV(LAST-2))
       GAMAV(LAST) = (FV(LAST) - (BETA * GAMAV(LAST-1)) - (AV(LAST) *
     &              GAMAV(LAST-2))) / MU

*      STORE SOLUTION OF SYSTEM IN VECTOR VV.

       VV(LAST) = GAMAV(LAST)
       VV(LAST-1) = GAMAV(LAST-1) - (DELTAV(LAST-1) * VV(LAST))
       DO 30 I = LAST-2, FIRST, -1
          VV(I) = GAMAV(I) - (DELTAV(I) * VV(I+1)) - (LAMDAV(I) *
     &    VV(I+2))
30     CONTINUE

*      CONVERT SOLUTION VECTOR BACK TO SINGLE-PRECISION.

       DO 40 K = FIRST, LAST
          SVV(K) = REAL(VV(K))
40     CONTINUE

       END

*****************************************************************
*                      THIRD LEVEL SUBROUTINES.                 *
*****************************************************************
*                                                               *
*  ROUTINE NAME  - BUILDM                                       *
*                                                               *
*  PURPOSE   - THIS SUBROUTINE IS USED TO GENERATE THE MATRIX OF *
*              LOCAL CELL MEMBRANE MODIFIED PHENOMENOLOGICAL    *
*              COEFFICIENTS (LM FORM) REQUIRED IN THE FLUX      *
*              CALCULATIONS.                                    *
*                                                               *
*  USAGE  - CALL BUILDM(MLMM)                                   *
*                                                               *
*  ARGUMENTS AND VARIABLES                                      *
*                                                               *
*             MLMM - MATRIX OF MODIFIED CELL MEMBRANE           *
*                    PHENOMENOLOGICAL COEFFICIENTS (LM FORM), IN *
*                    GMOLE CM-3 SEC-1.                          *
*                                                               *
*           TYPEFL - FLAG TO INDICATE WHICH ARITHMETIC MEAN     *
*                    CONCENTRATION NEEDS TO BE EVALUATED USING  *
*                    FUNCTION ARMEAN.                           *
*                                                               *
*           ARMEAN - FUNCTION SUBROUTINE USED TO EVALUATE THE I-TH *
*                    SOLUTE TRANSMEMBRANE ARITHMETIC MEAN       *
*                    CONCENTRATION, TYPEFL SET TO 1.            *
*                                                               *
*           AVCONC - AVERAGE CONCENTRATION VALUE TO BE USED TO  *
*                    GENERATE A MODIFIED COEFFICIENT (LM FORM), *
*                    IN GMOLE CM-3.                             *
*                                                               *
*  REMARKS   - THE PHENOMENOLOGICAL COEFFICIENTS ARE INPUT      *
*              INITIALLY AS PERMEABILITY COEFFICIENTS (P FORM)  *
*              AND ARE THEN MODIFIEDD (SEE SUBROUTINE MODCOF). FOR *
*              THE PURPOSE OF THE FLUX CALCULATIONS, IT IS      *
*              NECESSARY TO TRANSFORM THESE MODIFIED COEFFICIENTS *
*              TO THEIR TEMPERATURE AND CONCENTRATION DEPENDENT *
*              FORM (LM FORM). THIS PROCESS REQUIRES CONCENTRATIONS *
*              WITH DIMENSIONS (GMOLE CM-3).                    *
*                                                               *
*****************************************************************

*      DEBUG INIT, SUBTRACE
*      END DEBUG

       SUBROUTINE BUILDM(MLMM)

       INTEGER NSOL, NGRPTS, NCELLS

       PARAMETER(NSOL = 4, NGRPTS = 2500, NCELLS = 250)

*      ARGUMENTS.

       REAL*4 MLMM(NSOL+1, NSOL+1)

*      COMMON VARIABLES.

       COMMON /C1/ SOLNUM, IMPNUM, PCONUM, RNUM, RNUMEX, CELNUM,
     &             GRPCEL, COLNUM
       COMMON /C2/ PMVV(NSOL+1), RT
       COMMON /C3/ CSBOV(NSOL), CSCOV(NSOL), CSIOV(NSOL), CSRV(NSOL)
```

```
      COMMON /C10/ MPMV((NSOL+1)*(NSOL+2)/2),
     &             MPPV((NSOL+1)*(NSOL+2)/2)

      REAL*4 PMVV, RT, CSBOV, CSCOV, CSIOV, CSRV, MPMV, MPPV

      INTEGER SOLNUM, IMPNUM, PCONUM, RNUM, RNUMEX, CELNUM, GRPCEL,
     &        COLNUM

*     ROUTINE VARIABLES.

      REAL*4 AVCONC, ARMEAN

      INTEGER TYPEFL

*     SET ALL MLMM ELEMENTS TO 0.

      DO 10 I = 1, SOLNUM+1
      DO 10 J = 1, SOLNUM+1
         MLMM(I, J) = 0.0
10    CONTINUE

*     THEN FILL MLMM MATRIX WITH MODIFIED COEFFICIENTS (LM FORM).

*     NOTE THAT EACH PERMEABILITY COEF[illegible]ENT (P FORM) HAS
*     TO BE MULTIPLIED BY THE APPROPRI[illegible] AVERAGE CONCENTRATION
*     TO LEAD TO ITS CORRESPONDING CONCENTRATION DEPENDENT FORM.

      TYPEFL = 1

      DO 20 I = 1, SOLNUM-IMPNUM+1
      DO 20 J = 1, SOLNUM-IMPNUM+1

         IF (I .LT. J) THEN

           GO TO 20

          ELSE

           K = (I * (I - 1) / 2) + J

           IF ((I .EQ. 1) .AND. (J .EQ. 1)) THEN

             AVCONC = 1.0 / PMVV(1)

            ELSE IF ((J .EQ. 1) .AND. (I .NE. 1)) THEN

                   AVCONC =
     &               SQRT((CSRV(I-1) * ARMEAN(I-1, TYPEFL)) /
     &               PMVV(I))

                  ELSE IF ((J .NE. 1) .AND. (J .EQ. I)) THEN

                         AVCONC = CSRV(J-1) * ARMEAN(J-1, TYPEFL)

                        ELSE

                         AVCONC =
     &                     SQRT((CSRV(J-1) * ARMEAN(J-1, TYPEFL)) *
     &                     (CSRV(I-1) * ARMEAN(I-1, TYPEFL)))

           END IF

         END IF

         MLMM(I, J) = MPMV(K) * AVCONC
         MLMM(J, I) = MLMM(I, J)

20    CONTINUE

      END

*********************************************************************
*                                                                   *
*  ROUTINE NAME  - FGRADM                                           *
*                                                                   *
*  PURPOSE  - THIS SUBROUTINE EVALUATES THE LOCAL VALUES OF THE     *
*             DIFFERENCE GRADIENTS OF THERMODYNAMIC FORCES THAT     *
*             ARE RESPONSIBLE FOR THE OBSERVED FLUXES ACROSS THE    *
*             CELL MEMBRANE.                                        *
*                                                                   *
*  USAGE  - CALL FGRADM(FORCEV)                                     *
*                                                                   *
*  ARGUMENTS AND VARIABLES                                          *
*                                                                   *
*          FORCEV - VECTOR OF REDUCED THERMODYNAMIC FORCE           *
*                   DIFFERENCE GRADIENTS.                           *
*                                                                   *
*             SUM - SUMMATION VARIABLE.                             *
*                                                                   *
*           CTERM - TERM RELATED TO CONCENTRATION GRADIENT(S) IN    *
*                   CHEMICAL POTENTIAL GRADIENT, IN GMOLE CM-3.     *
*                                                                   *
*          T1, T2 - INTERMEDIATE VARIABLES USED IN CALCULATIONS.    *
*                                                                   *
*           PTERM - TERM RELATED TO HYDROSTATIC PRESSURE GRADIENT   *
*                   IN CHEMICAL POTENTIAL GRADIENT, IN GMOLE CM-3.  *
*                                                                   *
*          TYPEFL - FLAG TO INDICATE WHICH ARITHMETIC MEAN          *
*                   CONCENTRATION NEEDS TO BE EVALUATED USING       *
*                   FUNCTION ARMEAN.                                *
*                                                                   *
*          ARMEAN - FUNCTION SUBROUTINE USED TO EVALUATE THE I-TH   *
*                   SOLUTE TRANSMEMBRANE ARITHMETIC MEAN            *
*                   CONCENTRATION, TYPEFL SET TO 1.                 *
*                                                                   *
*  REMARKS  - SINCE THE MODEL SIMULATES THE BEHAVIOUR OF A PLANT    *
*             TISSUE, A HYDROSTATIC PRESSURE GRADIENT TERM IS       *
*             INCLUDED IN THE DEFINITION OF THE CHEMICAL POTENTIAL  *
*             GRADIENT OF ANY SPECIES.                              *
*                                                                   *
*             NOTE ALSO THAT IN THE CALCULATION OF THE FORCE        *
*             GRADIENTS, THE I-TH SOLUTE CHEMICAL POTENTIAL         *
*             GRADIENT IS EVALUATED ONLY IF THAT SPECIES IS AT      *
*             LEAST PRESENT ON ONE SIDE OF THE BARRIER. THIS IS     *
*             IMPORTANT SINCE THE ABSENCE OF THE I-TH SOLUTE        *
*             ON BOTH SIDES OF THE MEMBRANE IMPLIES THAT IT DOES    *
*             NOT EXIST THERMODYNAMICALLY AND THEREFORE CANNOT      *
*             BE PART OF THE ESTIMATION OF THE LOCAL FORCES.        *
*                                                                   *
```

```
************************************************************************

*     DEBUG INIT, SUBTRACE
*     END DEBUG

      SUBROUTINE FGRADM(FORCEV)

      INTEGER NSOL, NGRPTS, NCELLS

      PARAMETER(NSOL = 4, NGRPTS = 2600, NCELLS = 250)

*     ARGUMENTS.

      REAL*4 FORCEV(NSOL+1)

*     COMMON VARIABLES.

      COMMON /C1/ SOLNUM, IMPNUM, PCONUM, RNUM, RNUMEX, CELNUM,
     &            GRPCEL, COLNUM
      COMMON /C2/ PMVV(NSOL+1), RT
      COMMON /C3/ CSBOV(NSOL), CSCOV(NSOL), CSIOV(NSOL), CSRV(NSOL)
      COMMON /C7/ RNOVL, EMODFL, EMODCO(2), WRESF
      COMMON /C12/ CSIM(NSOL, 0:NGRPTS, 3), CSCM(NSOL, NCELLS, 3),
     &             CELLM(11, NCELLS, 3), MASSM(3, NSOL+1),
     &             STAGEV(NCELLS), CRITCV
      COMMON /C13/ NLEVEL, LVLCNT, GRPTI, CELLI

      REAL*4 PMVV, RT, CSBOV, CSCOV, CSIOV, CSRV, RNOVL,
     &    -   EMODCO, WRESF, CSIM, CSCM, CELLM, MASSM, CRITCV, NLEVEL

      INTEGER SOLNUM, IMPNUM, PCONUM, RNUM, RNUMEX, CELNUM, GRPCEL,
     &        COLNUM, EMODFL, STAGEV, LVLCNT, GRPTI, CELLI

*     ROUTINE VARIABLES.

      REAL*4 SUM, CTERM, T1, T2, PTERM, ARMEAN

      INTEGER TYPEFL

*     SET ALL ELEMENTS OF VECTOR FORCEV TO 0.

      DO 10 I = 1, SOLNUM+1
         FORCEV(I) = 0.0
10    CONTINUE

*     EVALUATE THE SOLVENT CHEMICAL POTENTIAL GRADIENT.

      TYPEFL = 1

*     1. TERM RELATED TO CONCENTRATION GRADIENT(S) (OSMOTIC
*        PRESSURE).

      SUM = 0.0
      DO 20 I = 1, SOLNUM
         IF ((CSIM(I, GRPTI, LVLCNT) .LE. 0.0) .AND.
     &       (CSCM(I, CELLI, LVLCNT) .LE. 0.0)) GO TO 20

         SUM = SUM + (CSRV(I) * (CSCM(I, CELLI, LVLCNT) -
     &                CSIM(I, GRPTI, LVLCNT)))
20    CONTINUE

      CTERM = SUM

*     2. TERM RELATED TO HYDROSTATIC PRESSURE GRADIENT.

      T1 = CSRV(SOLNUM) * (CSCOV(SOLNUM) - CSIOV(SOLNUM))

      IF (EMODFL .EQ. 0) THEN
        PTERM = ((EMODCO(1) / RT) * LOG(CELLM(1, CELLI, LVLCNT)))
     &          + T1
       ELSE
        T2 = EMODCO(2) / (RT * EMODCO(1))
        PTERM = ((T2 + T1) * (CELLM(1, CELLI, LVLCNT)**EMODCO(1)))
     &          - T2
      END IF

      IF (PTERM .GT. 0.0) STAGEV(CELLI) = 1

      IF (PTERM .LE. 0.0) PTERM = 0.0

      IF ((PTERM .EQ. 0.0) .AND. (STAGEV(CELLI) .EQ. 1))
     &             STAGEV(CELLI) = 2

      FORCEV(1) = PMVV(1) * (PTERM - CTERM)

*     EVALUATE EACH SOLUTE CHEMICAL POTENTIAL GRADIENT.

      DO 30 I = 1, SOLNUM

         IF ((CSIM(I, GRPTI, LVLCNT) .LE. 0.0) .AND.
     &       (CSCM(I, CELLI, LVLCNT) .LE. 0.0)) GO TO 30

         CTERM = (CSCM(I, CELLI, LVLCNT) - CSIM(I, GRPTI, LVLCNT)) /
     &           ARMEAN(I, TYPEFL)

         FORCEV(I+1) = CTERM + (PMVV(I+1) * PTERM)

30    CONTINUE

      END

************************************************************************
*                                                                      *
*  ROUTINE NAME  - BUILDP                                              *
*                                                                      *
*  PURPOSE  - THIS SUBROUTINE IS USED TO GENERATE THE MATRIX OF        *
*             THE MODIFIED PLASMODESMAL PHENOMENOLOGICAL               *
*             COEFFICIENTS (LP FORM) OF THE J, (J-1)-TH CELL           *
*             INTERFACE REQUIRED IN THE FLUX CALCULATIONS.             *
*                                                                      *
*  USAGE  - CALL BUILDP(MLPM)                                          *
*                                                                      *
*  ARGUMENTS AND VARIABLES                                             *
*                                                                      *
*            MLPM - MATRIX OF MODIFIED PLASMODESMAL                    *
*                   PHENOMENOLOGICAL COEFFICIENTS (LP FORM), IN        *
*                   GMOLE CM-3 SEC-1.                                  *
*                                                                     *
*       TYPEFL  - FLAG TO INDICATE WHICH ARITHMETIC MEAN              *
*                 CONCENTRATION NEEDS TO BE EVALUATED USING           *
*                 FUNCTION ARMEAN.                                    *
*                                                                     *
*       ARMEAN  - FUNCTION SUBROUTINE USED TO EVALUATE THE J,         *
*                 (J-1)-TH CELL INTERFACE ARITHMETIC MEAN             *
*                 CONCENTRATION. IF SURFACE CELL TYPEFL IS SET        *
*                 TO 3, OTHERWISE TYPEFL IS SET TO 2.                 *
*                                                                     *
*       AVCONC  - AVERAGE CONCENTRATION VALUE TO BE USED TO           *
*                 GENERATE A MODIFIED COEFFICIENT (LP FORM),          *
*                 IN GMOLE CM-3.                                      *
*                                                                     *
* REMARKS - THE PHENOMENOLOGICAL COEFFICIENTS ARE INPUT               *
*           INITIALLY AS PERMEABILITY COEFFICIENTS (P FORM)           *
*           AND ARE THEN MODIFIEDD (SEE SUBROUTINE MODCOF). FOR       *
*           THE PURPOSE OF THE FLUX CALCULATIONS, IT IS               *
*           NECESSARY TO TRANSFORM THESE MODIFIED COEFFICIENTS        *
*           TO THEIR TEMPERATURE AND CONCENTRATION DEPENDENT          *
*           FORM (LP FORM). THIS PROCESS REQUIRES CONCENTRATIONS      *
*           WITH DIMENSIONS (GMOLE CM-3).                             *
*                                                                     *
***********************************************************************

*     DEBUG INIT, SUBTRACE
*     END DEBUG

      SUBROUTINE BUILDP(MLPM)

      INTEGER NSOL, NGRPTS, NCELLS

      PARAMETER(NSOL = 4, NGRPTS = 2500, NCELLS = 250)

*     ARGUMENTS.

      REAL*4 MLPM(NSOL+1, NSOL+1)

*     COMMON VARIABLES.

      COMMON /C1/ SOLNUM, IMPNUM, PCONUM, RNUM, RNUMEX, CELNUM,
     &            GRPCEL, COLNUM
      COMMON /C2/ PMVV(NSOL+1), RT
      COMMON /C3/ CSBOV(NSOL), CSCOV(NSOL), CSIOV(NSOL), CSRV(NSOL)
      COMMON /C10/ MPMV((NSOL+1)*(NSOL+2)/2),
     &             MPPV((NSOL+1)*(NSOL+2)/2)

      REAL*4 PMVV, RT, CSBOV, CSCOV, CSIOV, CSRV, MPMV, MPPV

      INTEGER SOLNUM, IMPNUM, PCONUM, RNUM, RNUMEX, CELNUM, GRPCEL,
     &        COLNUM

*     ROUTINE VARIABLES.

      REAL*4 AVCONC, ARMEAN

      INTEGER TYPEFL

*     SET ALL MLPM ELEMENTS TO 0.

      DO 10 I = 1, SOLNUM+1
      DO 10 J = 1, SOLNUM+1
        MLPM(I, J) = 0.0
10    CONTINUE

*     THEN FILL MLPM MATRIX WITH MODIFIED COEFFICIENTS (LP FORM).

*     NOTE THAT EACH PERMEABILITY COEFFICIENT (P FORM) HAS
*     TO BE MULTIPLIED BY THE APPROPRIATE AVERAGE CONCENTRATION
*     TO LEAD TO ITS CORRESPONDING CONCENTRATION DEPENDENT FORM.

      IF (CELLI .EQ. 1) THEN
        TYPEFL = 3
      ELSE
        TYPEFL = 2
      END IF

      DO 20 I = 1, SOLNUM-IMPNUM+1
      DO 20 J = 1, SOLNUM-IMPNUM+1

         IF (I .LT. J) THEN

           GO TO 20

         ELSE

           K = (I * (I - 1) / 2) + J

           IF ((I .EQ. 1) .AND. (J .EQ. 1)) THEN

             AVCONC = 1.0 / PMVV(1)

            ELSE IF ((J .EQ. 1) .AND. (I .NE. 1)) THEN

                   AVCONC =
     &              SQRT((CSRV(I-1) * ARMEAN(I-1, TYPEFL)) /
     &              PMVV(1))

                  ELSE IF ((J .NE. 1) .AND. (J .EQ. I)) THEN

                         AVCONC = CSRV(J-1) * ARMEAN(J-1, TYPEFL)

                        ELSE

                         AVCONC =
     &                    SQRT((CSRV(J-1) * ARMEAN(J-1, TYPEFL)) *
     &                    (CSRV(I-1) * ARMEAN(I-1, TYPEFL)))

          END IF

        END IF

        MLPM(I, J) = MPPV(K) * AVCONC
        MLPM(J, I) = MLPM(I, J)

20    CONTINUE

      END
```

```
*************************************************************************
*                                                                       *
*   ROUTINE NAME  - FGRADP                                              *
*                                                                       *
*   PURPOSE  - THIS SUBROUTINE EVALUATES THE LOCAL VALUES OF THE        *
*              DIFFERENCE GRADIENTS OF THERMODYNAMIC FORCES THAT        *
*              ARE RESPONSIBLE FOR THE OBSERVED FLUXES ACROSS THE       *
*              J, (J-1)-TH CELL INTERFACE (PLASMODESMAL FLUXES).        *
*                                                                       *
*   USAGE  - CALL FGRADP(FORCEV)                                        *
*                                                                       *
*   ARGUMENTS AND VARIABLES                                             *
*                                                                       *
*          FORCEV - VECTOR OF REDUCED THERMODYNAMIC FORCE               *
*                   DIFFERENCE GRADIENTS.                               *
*                                                                       *
*             SUM - SUMMATION VARIABLE.                                 *
*                                                                       *
*           CTERM - TERM RELATED TO CONCENTRATION GRADIENT(S) IN        *
*                   CHEMICAL POTENTIAL GRADIENT, IN GMOLE CM-3.         *
*                                                                       *
*          T1, T2 - INTERMEDIATE VARIABLES USED IN CALCULATIONS.        *
*                                                                       *
*        PJ, PJM1 - HYDROSTATIC PRESSURE IN THE J-TH AND (J-1)-TH       *
*                   CELL RESPECTIVELY, IN GMOLE CM-3.                   *
*                                                                       *
*           PTERM - TERM RELATED TO HYDROSTATIC PRESSURE GRADIENT       *
*                   IN CHEMICAL POTENTIAL GRADIENT, IN GMOLE CM-3.      *
*                                                                       *
*          TYPEFL - FLAG TO INDICATE WHICH ARITHMETIC MEAN              *
*                   CONCENTRATION NEEDS TO BE EVALUATED USING           *
*                   FUNCTION ARMEAN.                                    *
*                                                                       *
*          ARMEAN - FUNCTION SUBROUTINE USED TO EVALUATE THE J,         *
*                   (J-1)-TH CELL INTERFACE ARITHMETIC MEAN             *
*                   CONCENTRATION. IF SURFACE CELL TYPEFL IS SET        *
*                   TO 3, OTHERWISE TYPEFL IS SET TO 2.                 *
*                                                                       *
*   REMARKS  - SINCE THE MODEL SIMULATES THE BEHAVIOUR OF A PLANT       *
*              TISSUE, A HYDROSTATIC PRESSURE GRADIENT TERM IS          *
*              INCLUDED IN THE DEFINITION OF THE CHEMICAL POTENTIAL     *
*              GRADIENT OF ANY SPECIES.                                 *
*                                                                       *
*              NOTE ALSO THAT IN THE CALCULATION OF THE FORCE           *
*              GRADIENTS, THE I-TH SOLUTE CHEMICAL POTENTIAL            *
*              GRADIENT IS EVALUATED ONLY IF THAT SPECIES IS AT         *
*              LEAST PRESENT ON ONE SIDE OF THE BARRIER. THIS IS        *
*              IMPORTANT SINCE THE ABSENCE OF THE I-TH SOLUTE           *
*              ON BOTH SIDES OF THE MEMBRANE IMPLIES THAT IT DOES       *
*              NOT EXIST THERMODYNAMICALLY AND THEREFORE CANNOT         *
*              BE PART OF THE ESTIMATION OF THE LOCAL FORCES.           *
*                                                                       *
*************************************************************************

*     DEBUG INIT, SUBTRACE
*     END DEBUG

      SUBROUTINE FGRADP(FORCEV)

      INTEGER NSOL, NGRPTS, NCELLS

      PARAMETER(NSOL = 4, NGRPTS = 2500, NCELLS = 250)

*     ARGUMENTS.

      REAL*4 FORCEV(NSOL+1)

*     COMMON VARIABLES.

      COMMON /C1/ SOLNUM, IMPNUM, PCONUM, RNUM, RNUMEX, CELNUM,
     &            GRPCEL, COLNUM
      COMMON /C2/ PMVV(NSOL+1), RT
      COMMON /C3/ CSBOV(NSOL), CSCOV(NSOL), CSIOV(NSOL), CSRV(NSOL)
      COMMON /C7/ RNOVL, EMODFL, EMODCO(2), WRESF
      COMMON /C12/ CSIM(NSOL, 0:NGRPTS, 3), CSCM(NSOL, NCELLS, 3),
     &             CELLM(11, NCELLS, 3), MASSM(3, NSOL+1),
     &             STAGEV(NCELLS), CRITCV
      COMMON /C13/ NLEVEL, LVLCNT, GRPTI, CELLI

      REAL*4 PMVV, RT, CSBOV, CSCOV, CSIOV, CSRV, RNOVL,
     &       EMODCO, WRESF, CSIM, CSCM, CELLM, MASSM, CRITCV, NLEVEL

      INTEGER SOLNUM, IMPNUM, PCONUM, RNUM, RNUMEX, CELNUM, GRPCEL,
     &        COLNUM, EMODFL, STAGEV, LVLCNT, GRPTI, CELLI

*     ROUTINE VARIABLES.

      REAL*4 SUM, CTERM, T1, T2, PJ, PJM1, PTERM, ARMEAN

      INTEGER TYPEFL

*     SET ALL ELEMENTS OF VECTOR FORCEV TO 0.

      DO 10 I = 1, SOLNUM+1
         FORCEV(I) = 0.0
10    CONTINUE

*     EVALUATE THE SOLVENT CHEMICAL POTENTIAL GRADIENT.

*     1. TERM RELATED TO CONCENTRATION GRADIENT(S) (OSMOTIC
*        PRESSURE).

      SUM = 0.0
      IF (CELLI .EQ. 1) THEN

        DO 20 I = 1, SOLNUM

          IF ((CSCM(I, CELLI, LVLCNT) .LE. 0.0) .AND.
     &        (CSBOV(I) .LE. 0.0)) GO TO 20

          SUM = SUM + (CSRV(I) * (CSCM(I, CELLI, LVLCNT) -
     &                 CSBOV(I)))

20      CONTINUE

      ELSE

        DO 30 I = 1, SOLNUM
```

```
            IF ((CSCM(I, CELLI, LVLCNT) .LE. 0.0) .AND.
     &          (CSCM(I, CELLI-1, LVLCNT) .LE. 0.0)) GO TO 30

            SUM = SUM + (CSRV(I) * (CSCM(I, CELLI, LVLCNT) -
     &                   CSCM(I, CELLI-1, LVLCNT)))

30        CONTINUE

        END IF

        CTERM = SUM

*       2. TERM RELATED TO HYDROSTATIC PRESSURE GRADIENT.

        T1 = CSRV(SOLNUM) * (CSCOV(SOLNUM) - CSIOV(SOLNUM))

        IF (STAGEV(CELLI) .GE. 2) THEN

          PJ = 0.0

         ELSE

          IF (EMODFL .EQ. 0) THEN
            PJ = ((EMODCO(1) / RT) * LOG(CELLM(1, CELLI, LVLCNT)))
     &           + T1
           ELSE
            T2 = EMODCO(2) / (RT * EMODCO(1))
            PJ = ((T2 + T1) * (CELLM(1, CELLI, LVLCNT)**EMODCO(1)))
     &           - T2
          END IF

        END IF

        IF ((STAGEV(CELLI-1) .GE. 2) .OR. (CELLI-1 .EQ. 0)) THEN

          PJM1 = 0.0

         ELSE

          IF (EMODFL .EQ. 0) THEN
            PJM1 = ((EMODCO(1) / RT) * LOG(CELLM(1, CELLI-1, LVLCNT)))
     &           + T1
           ELSE
            T2 = EMODCO(2) / (RT * EMODCO(1))
            PJM1 = ((T2 + T1) * (CELLM(1,CELLI-1, LVLCNT)**EMODCO(1)))
     &           - T2
          END IF

        END IF

        PTERM = PJ - PJM1

        FORCEV(1) = PMVV(1) * (PTERM - CTERM)

*       EVALUATE EACH SOLUTE CHEMICAL POTENTIAL GRADIENT.

        DO 40 I = 1, SOLNUM

            IF (CELLI .EQ. 1) THEN

              TYPEFL = 3

              IF ((CSCM(I, CELLI, LVLCNT) .LE. 0.0) .AND.
     &            (CSBOV(I) .LE. 0.0)) GO TO 40

              CTERM = (CSCM(I, CELLI, LVLCNT) - CSBOV(I)) /
     &                 ARMEAN(I, TYPEFL)

             ELSE

              TYPEFL = 2

              IF ((CSCM(I, CELLI, LVLCNT) .LE. 0.0) .AND.
     &            (CSCM(I, CELLI-1, LVLCNT) .LE. 0.0)) GO TO 40

              CTERM =
     &         (CSCM(I, CELLI, LVLCNT) - CSCM(I, CELLI-1, LVLCNT)) /
     &         ARMEAN(I, TYPEFL)

            END IF

            FORCEV(I+1) = CTERM + (PMVV(I+1) * PTERM)

40      CONTINUE

        END

***********************************************************************
*                                                                     *
*  ROUTINE NAME  - TAFUNC                                             *
*                                                                     *
*  PURPOSE  - THIS SUBROUTINE EVALUATES THE CURRENT J-TH CELL         *
*             REDUCED TIME DERIVATIVES OF PLASMALEMMAL AND            *
*             PLASMODESMAL TRANSFER AREAS.                            *
*                                                                     *
*  USAGE  - CALL TAFUNC(CVTDM, TATDM)                                 *
*                                                                     *
*  ARGUMENTS AND VARIABLES                                            *
*                                                                     *
*           CVTDM - MATRIX OF UNIT CELL VOLUME TIME DERIVATIVES,      *
*                   IN SEC-1.                                         *
*                                                                     *
*           TATDM - MATRIX OF UNIT CELL TRANSFER AREA TIME            *
*                   DERIVATIVES, IN SEC-1.                            *
*                                                                     *
***********************************************************************

*       DEBUG INIT, SUBTRACE
*       END DEBUG

        SUBROUTINE TAFUNC(CVTDM, TATDM)

        INTEGER NSOL, NORPTS, NCELLS

        PARAMETER(NSOL = 4, NORPTS = 2500, NCELLS = 250)

*       ARGUMENTS.
```

```
      REAL*4 CVTDM(3, NCELLS), TATDM(3, NCELLS)

*     COMMON VARIABLES.

      COMMON /C12/ CSIM(NSOL, 0:NGRPTS, 3), CSCM(NSOL, NCELLS, 3),
     &             CELLM(11, NCELLS, 3), MASSM(3, NSOL+1),
     &             STAGEV(NCELLS), CRITCV
      COMMON /C13/ NLEVEL, LVLCNT, GRPTI, CELLI

      REAL*4 CSIM, CSCM, CELLM, MASSM, CRITCV, NLEVEL

      INTEGER STAGEV, LVLCNT, GRPTI, CELLI

*     EVALUATE PLASMALEMMAL AND PLASMODESMAL TRANSFER AREA TIME
*     DERIVATIVES.

      IF (STAGEV(CELLI) .EQ. 1) THEN
        TATDM(2, CELLI) =
     &    (2.0 / 3.0) * (CELLM(1, CELLI, LVLCNT)**(- 1.0 / 3.0)) *
     &    CVTDM(1, CELLI)
       ELSE
        TATDM(2, CELLI) = 0.0
      END IF

      TATDM(3, CELLI) = TATDM(2, CELLI)

      END

******************************************************************
*                                                                *
*  ROUTINE NAME  - GPFUNC                                        *
*                                                                *
*  PURPOSE  - THIS SUBROUTINE EVALUATES, FOR EACH CELL, THE      *
*             CURRENT TIME DERIVATIVES OF THE REDUCED CELL LENGTH, *
*             CELL INTERSTITIUM, CELLULAR AND BUFFER RADII, OF THE *
*             APPARENT INTERSTITIUM RESISTANCE FACTOR AND OF THE *
*             APOPLASTIC TRANSFER AREA.                          *
*                                                                *
*  USAGE  - CALL GPFUNC(CVTDM, TATDM, GPTDM)                     *
*                                                                *
*  ARGUMENTS AND VARIABLES                                       *
*                                                                *
*          CVTDM - MATRIX OF UNIT CELL VOLUME TIME DERIVATIVES,  *
*                  IN SEC-1                                      *
*                                                                *
*          TATDM - MATRIX OF UNIT CELL TRANSFER AREA TIME        *
*                  DERIVATIVES, IN SEC-1                         *
*                                                                *
*          GPTDM - MATRIX OF UNIT CELL GEOMETRICAL PARAMETER     *
*                  TIME DERIVATIVES, IN SEC-1.                   *
*                                                                *
*             PI - PI CONSTANT (DIMENSIONLESS).                  *
*                                                                *
* T1, T2, T3, T4, - INTERMEDIATE VARIABLES USED IN CALCULATIONS  *
* T5                                                             *
*                                                                *
******************************************************************

*     DEBUG INIT, SUBTRACE
*     END DEBUG

      SUBROUTINE GPFUNC(CVTDM, TATDM, GPTDM)

      INTEGER NSOL, NGRPTS, NCELLS

      PARAMETER(NSOL = 4, NGRPTS = 2500, NCELLS = 250)

*     ARGUMENTS.

      REAL*4 CVTDM(3, NCELLS), TATDM(3, NCELLS), GPTDM(5, NCELLS)

*     COMMON VARIABLES.

      COMMON /C5/ SPVFO, WVFO, VOIDFO, VCO, VIO, VTO, AIO, ACMO,
     &            ACPO, LCO, RIO, RCO, RBO
      COMMON /C7/ RNOVL, EMODFL, EMODCO(2), WRESF
      COMMON /C12/ CSIM(NSOL, 0:NGRPTS, 3), CSCM(NSOL, NCELLS, 3),
     &             CELLM(11, NCELLS, 3), MASSM(3, NSOL+1),
     &             STAGEV(NCELLS), CRITCV
      COMMON /C13/ NLEVEL, LVLCNT, GRPTI, CELLI

      REAL*4 SPVFO, WVFO, VOIDFO, VCO, VIO, VTO, AIO, ACMO, ACPO,
     &       LCO, RIO, RCO, RBO, RNOVL, EMODCO, WRESF, CSIM, CSCM,
     &       CELLM, MASSM, CRITCV, NLEVEL

      INTEGER EMODFL, STAGEV, LVLCNT, GRPTI, CELLI

*     ROUTINE VARIABLES.

      REAL*4 PI, T1, T2, T3, T4, T5

*     EVALUATE CELL LENGTH TIME DERIVATIVE.

      GPTDM(1,CELLI)=(1.0/(3.0*(CELLM(3,CELLI,LVLCNT)**(2.0/3.0))))
     &                * CVTDM(3, CELLI)

*     EVALUATE INTERSTITIUM CYLINDER RADIUS TIME DERIVATIVE.

      GPTDM(2, CELLI) =
     &  - ((CELLM(5, CELLI, LVLCNT) / (CELLM(7, CELLI, LVLCNT)**2))
     &  * GPTDM(1, CELLI)) + ((1.0 / CELLM(7, CELLI, LVLCNT)) *
     &  TATDM(2, CELLI))

*     EVALUATE CELLULAR CYLINDER RADIUS TIME DERIVATIVE.

      DATA PI/3.14159/

      T1 = VCO / (PI * (RCO**2) * LCO)
      T2 = - CELLM(1, CELLI, LVLCNT) / (2.0 *
     &     CELLM(9, CELLI, LVLCNT) * (CELLM(7, CELLI, LVLCNT)**2))
      T3 = 1.0 / (2.0 * CELLM(9, CELLI, LVLCNT) *
     &     CELLM(7, CELLI, LVLCNT))
      T4 = (RIO / RCO)**2
      T5 = CELLM(8, CELLI, LVLCNT) / CELLM(9, CELLI, LVLCNT)

      GPTDM(3, CELLI) = (T1 * ((T2 * GPTDM(1, CELLI)) +
     &                  (T3 * CVTDM(1, CELLI)))) +
      &                   (T4 * T5 * GPTDM(2, CELLI))
*     EVALUATE BUFFER CYLINDER RADIUS TIME DERIVATIVE.

      T1 = VIO / (PI * (RBO**2) * LCO)
      T2 = - CELLM(2, CELLI, LVLCNT) / (2.0 *
     &     CELLM(10, CELLI, LVLCNT) * (CELLM(7, CELLI, LVLCNT)**2))
      T3 = 1.0 / (2.0 * CELLM(10, CELLI, LVLCNT) *
     &     CELLM(7, CELLI, LVLCNT))
      T4 = (RIO / RBO)**2
      T5 = CELLM(8, CELLI, LVLCNT) / CELLM(10, CELLI, LVLCNT)

      GPTDM(4, CELLI) = - (T1 * ((T2 * GPTDM(1, CELLI)) +
     &                   (T3 * CVTDM(2, CELLI)))) +
     &                   (T4 * T5 * GPTDM(2, CELLI))

*     EVALUATE TIME DERIVATIVE OF APPARENT INTERSTITIUM
*     RESISTANCE FACTOR.

      GPTDM(5, CELLI) = (1.0 - WRESF) * (WVFO / VOIDFO) *
     &  (1.0 / (CELLM(2, CELLI, LVLCNT)**2)) * CVTDM(2, CELLI)

*     FINALLY, EVALUATE TIME DERIVATIVE OF APOPLASTIC TRANSFER AREA.

      TATDM(1, CELLI) = (2.0 * PI / AIO) *
     &  (((RIO**2) * CELLM(8, CELLI, LVLCNT) * GPTDM(2, CELLI)) -
     &   ((RBO**2) * CELLM(10, CELLI, LVLCNT) * GPTDM(4, CELLI)))

      END

*******************************************************************
*                                                                 *
*  ROUTINE NAME  - CCFUNC                                         *
*                                                                 *
*  PURPOSE  - THIS SUBROUTINE EVALUATES THE CURRENT J-TH CELL     *
*             REDUCED SOLUTE CONCENTRATION TIME DERIVATIVES. SINCE *
*             THERE MIGHT BE A CERTAIN AMOUNT OF NON-OSMOTIC      *
*             VOLUME IN THE CELL, THE SOLUTION VOLUME IS LESS THAN *
*             THE CELL VOLUME BY AN AMOUNT OF DEAD SPACE RNOVL.   *
*                                                                 *
*  USAGE  - CALL CCFUNC(FLUXMM, FLUXPM, CVTDM, CSTDM)             *
*                                                                 *
*  ARGUMENTS AND VARIABLES                                        *
*                                                                 *
*          FLUXMM - MATRIX OF MODIFIED TRANSMEMBRANE FLUXES, IN   *
*                   GMOLE CM-3 SEC-1.                             *
*                                                                 *
*          FLUXPM - MATRIX OF MODIFIED PLASMODESMAL FLUXES, IN    *
*                   GMOLE CM-3 SEC-1.                             *
*                                                                 *
*           CVTDM - MATRIX OF UNIT CELL VOLUME TIME DERIVATIVES,  *
*                   IN SEC-1.                                     *
*                                                                 *
*           CSTDM - MATRIX OF CELL SOLUTE CONCENTRATION TIME      *
*                   DERIVATIVES, IN SEC-1.                        *
*                                                                 *
*          STARTX - GRID POINT POSITION INDEX ON THE EXTENDED     *
*                   SPACE GRID.                                   *
*                                                                 *
*             SUM - SUMMATION VARIABLE.                           *
*                                                                 *
* X, Y, NX, C, IC - VARIABLES USED IN SUBROUTINES IQHSCU AND      *
* A, B, Q, IER      DCSQDU. SEE IMSL LIBRARY ROUTINE DESCRIPTIONS *
*                   FOR DETAILS.                                  *
*                                                                 *
*  MINPM1, MINPP1 - VALUES OF PLASMODESMAL TRANSFER AREA TO BE    *
*                   USED IN CALCULATION OF CELLULAR VOLUME TIME   *
*                   DERIVATIVE (DIMENSIONLESS).                   *
*                                                                 *
*  REMARKS  - THIS SUBROUTINE USES A QUASI-CUBIC HERMITE          *
*             INTERPOLATION PROCEDURE TO GENERATE SPLINE          *
*             COEFFICIENTS THAT ARE USED, THROUGH A CUBIC SPLINE  *
*             QUADRATURE ALGORITHM, TO ESTIMATE THE INTEGRAL OF   *
*             THE LOCAL TRANSMEMBRANE SOLUTE FLUXES OVER THE      *
*             LENGTH OF THE CELL (SEE IMSL LIBRARY                *
*             FOR DETAILS ON ROUTINE IQHSCU AND DCSQDU).          *
*                                                                 *
*******************************************************************

*     DEBUG INIT, SUBTRACE
*     END DEBUG

      SUBROUTINE CCFUNC(FLUXMM, FLUXPM, CVTDM, CSTDM)

      INTEGER NSOL, NGRPTS, NCELLS

      PARAMETER(NSOL = 4, NGRPTS = 2500, NCELLS = 250)

*     ARGUMENTS.

      REAL*4 FLUXMM(NSOL+1, 0:NGRPTS), FLUXPM(NSOL+1, NCELLS, 3),
     &       CVTDM(3, NCELLS), CSTDM(NSOL, NCELLS)

*     COMMON VARIABLES.

      COMMON /C1/ SOLNUM, IMPNUM, PCONUM, RNUM, RNUMEX, CELNUM,
     &            GRPCEL, COLNUM
      COMMON /C3/ CSSOV(NSOL), CSCOV(NSOL), CSIOV(NSOL), CSRV(NSOL)
      COMMON /C7/ RNOVL, EMODFL, EMODCO(2), WRESF
      COMMON /C8/ NSTEPV(NCELLS), KSTEP
      COMMON /C12/ CSIM(NSOL, 0:NGRPTS, 3), CSCM(NSOL, NCELLS, 3),
     &             CELLM(11, NCELLS, 3), MASSM(3, NSOL+1),
     &             STAGEV(NCELLS), CRITCV
      COMMON /C13/ NLEVEL, LVLCNT, GRPTI, CELLI

      REAL*4 CSSOV, CSCOV, CSIOV, CSRV, RNOVL, EMODCO, WRESF,
     &       NSTEPV, KSTEP, CSIM, CSCM, CELLM, MASSM, CRITCV, NLEVEL

      INTEGER SOLNUM, IMPNUM, PCONUM, RNUM, RNUMEX, CELNUM, GRPCEL,
     &        COLNUM, EMODFL, STAGEV, LVLCNT, GRPTI, CELLI

*     ROUTINE VARIABLES.

      REAL*4 SUM, X(NGRPTS/NCELLS), Y(NGRPTS/NCELLS),
     &       C(NGRPTS/NCELLS, 3), A, B, Q, MINPM1, MINPP1

*     INTEGER STARTX, NX, IC, IER
*     EVALUATE THE J-TH CELL SOLUTE CONCENTRATION GRADIENTS.

      STARTX = RNUMEX - (CELLI * GRPCEL)
      SUM = 0.0
      DO 10 K = CELNUM, CELLI+1, -1
         SUM = SUM + CELLM(7, K, LVLCNT)
10    CONTINUE

      X(1) = SUM
      DO 20 K = 1, GRPCEL-1
         X(K+1) = X(K) + HSTEPV(CELLI)
20    CONTINUE

      DO 30 I = 1, SOLNUM

*        USING QUASI-HERMITE SPLINE, GENERATE MATRIX OF SPLINE
*        COEFFICIENTS.

         DO 40 K = 1, GRPCEL
            Y(K) = FLUXMM(I+1, STARTX+K)
40       CONTINUE

         NX = GRPCEL
         IC = NGRPTS / NCELLS

         CALL IQHSCU(X, Y, NX, C, IC, IER)

*        INTEGRATE THE SPLINE OVER THE REQUIRED INTERVAL.

         A = X(1)
         B = X(GRPCEL)

         CALL DCSQDU(X, Y, NX, C, IC, A, B, Q, IER)

*        ESTIMATE THE I-TH SOLUTE CONCENTRATION TIME DERIVATIVE.

         IF (CELLI .EQ. 1) THEN
           MINPM1 = CELLM(6, CELLI, LVLCNT)
          ELSE
           MINPM1 =
     &      MIN(CELLM(6, CELLI, LVLCNT), CELLM(6, CELLI-1, LVLCNT))
         END IF

         IF (CELLI .EQ. CELNUM) THEN
           MINPP1 = CELLM(6, CELLI, LVLCNT)
          ELSE
           MINPP1 =
     &      MIN(CELLM(6, CELLI, LVLCNT), CELLM(6, CELLI+1, LVLCNT))
         END IF

         CSTDM(I, CELLI) = (1.0 / (CELLM(1, CELLI, LVLCNT) - RNOVL))
     &                 * ((CELLM(8, CELLI, LVLCNT) * Q / CSRV(I)) +
     &                 (MINPM1 * FLUXPM(I+1, CELLI, 1) / CSRV(I)) +
     &                 (MINPP1 * FLUXPM(I+1, CELLI, 2) / CSRV(I)) -
     &                 (CSCM(I, CELLI, LVLCNT) * CVTDM(1, CELLI)))

30    CONTINUE

      END

*******************************************************************
*                  FOURTH LEVEL SUBROUTINES.                      *
*******************************************************************
*                                                                 *
*  ROUTINE NAME  - ARMEAN                                         *
*                                                                 *
*  PURPOSE   - THIS FUNCTION SUBROUTINE EVALUATES THE REDUCED I-TH *
*              SOLUTE ARITHMETIC MEAN CONCENTRATION FROM THE      *
*              REDUCED CONCENTRATION VALUES ON BOTH SIDES OF THE  *
*              BARRIER.                                           *
*                                                                 *
*  USAGE   - XYZ = ARMEAN(SOLI, TYPEFL)                           *
*                                                                 *
*  ARGUMENTS AND VARIABLES                                        *
*                                                                 *
*              SOLI - SOLUTE INDEX.                               *
*                                                                 *
*            TYPEFL - FLAG TO INDICATE WHICH ARITHMETIC MEAN      *
*                     CONCENTRATION NEEDS TO BE EVALUATED.        *
*                                                                 *
*                     TYPEFL = 1 : TRANSMEMBRANE MEAN             *
*                                  CONCENTRATION.                 *
*                     TYPEFL = 2 : J-(J-1)-TH CELL INTERFACE      *
*                                  MEAN CONCENTRATION.            *
*                     TYPEFL = 3 : OSMOTICUM-FIRST CELL INTERFACE *
*                                  MEAN CONCENTRATION.            *
*                                                                 *
*******************************************************************

*     DEBUG INIT, SUBTRACE
*     END DEBUG

      REAL FUNCTION ARMEAN*4(SOLI, TYPEFL)

      INTEGER NSOL, NGRPTS, NCELLS

      PARAMETER(NSOL = 4, NGRPTS = 2500, NCELLS = 250)

*     ARGUMENTS.

      INTEGER SOLI, TYPEFL

*     COMMON VARIABLES.

      COMMON /C3/ CSBOV(NSOL), CSCOV(NSOL), CSIOV(NSOL), CSRV(NSOL)
      COMMON /C12/ CSIM(NSOL, 0:NGRPTS, 3), CSCM(NSOL, NCELLS, 3),
     &             CELLM(11, NCELLS, 3), MASSM(3, NSOL+1),
     &             STAGEV(NCELLS), CRITCV
      COMMON /C13/ NLEVEL, LVLCNT, GRPTI, CELLI

      REAL*4 CSBOV, CSCOV, CSIOV, CSRV, CSIM, CSCM, CELLM,
     &       MASSM, CRITCV, NLEVEL

      INTEGER STAGEV, LVLCNT, GRPTI, CELLI

*     DUE TO FLUCTUATIONS INHERENT TO THE NUMERICAL TECHNIQUES
*     USED IN THIS PROGRAM, THE VALUE OF ARMEAN IS FORCED TO BE
*     GREATER OR EQUAL TO 0 TO PROHIBIT ANY OCCURRENCE OF NEGATIVE
*     AVERAGE CONCENTRATIONS FOR LIMITING VALUES.

      IF (TYPEFL .EQ. 1) THEN

         ARMEAN = ABS(
&          (CSCM(SOLI, CELLI, LVLCNT) + CSIM(SOLI, GRPTI, LVLCNT)) /
&           2.0)

       ELSE IF (TYPEFL .EQ. 2) THEN

              ARMEAN = ABS((CSCM(SOLI, CELLI, LVLCNT) +
&                      CSCM(SOLI, CELLI-1, LVLCNT)) / 2.0)

           ELSE

              ARMEAN = ABS((CSCM(SOLI,CELLI,LVLCNT)+CSBOV(SOLI))/
&                      2.0)

      END IF

      END
```

17. APPENDIX 6: RESUME

Ce travail retrace les étapes nécessaires à la mise au point d'un modèle mathématique décrivant les phénomènes de transfert de masse dans des tissus soumis à une déshydratation osmotique. On a prêté une attention particulière aux paramètres du modèle afin d'inclure une mesure de la résistance des membranes cellulaires au transfert de masse, sous forme de coefficients macroscopiques de perméabilité. Pour ce faire, on a fait appel à la théorie de la thermodynamique du non-équilibre pour développer les équations phénoménologiques nécessaires à la description du transport de l'eau et de solutés dans les structures cellulaires étudiées. La forme plus fondamentale de ces relations, habituellement appelée *forme fondamentale d'Onsager*, a été préférée à la forme "pratique" utilisée par la majorité des chercheurs.

Un programme d'optimisation, reposant sur une version modifiée de la méthode du "Simplex" et basé sur la modélisation et la simulation du comportement de suspensions cellulaires soumises à une déshydratation osmotique, a permis d'estimer statistiquement les coefficients macroscopiques de perméabilité de ces populations. L'influence de la température et de la concentration sur ces paramètres a pu ainsi être étudiée.

S'appuyant sur le formalisme théorique utilisé pour décrire le comportement de cellules en suspension, un modèle plus global, intégrant les caractéristiques de la matrice

support, a été écrit pour simuler le transport isotherme de matière dans des structures cellulaires complexes. Le modèle se compose de deux parties principales: (1) la description de l'interaction des cellules avec leur environnement immédiat, prise en charge par les relations dérivées de la théorie de la thermodynamique du non-équilibre, et (2) la description des phénomènes de diffusion dans la structure elle-même, représentée par des équations fickiennes du second-ordre. Due à la complexité de la structure des tissus considérés, une matrice cellulaire hypothétique basée sur l'emploi de l'artifice mathématique de la "cellule cylindrique équivalente" a été utilisée pour les simulations. Le modèle, testé dans l'hypothèse d'un milieu géométriquement simple, a permis de simuler le mouvement de l'eau et de solutés perméables et imperméables dans ces tissus. Des études paramétriques ont permis d'examiner l'influence de certaines propriétés des tissus cellulaires considérés sur la dynamique des phénomènes de transfert de masse se produisant dans ces structures.

18. VITA

Biographical data

Name:	Christian Toupin
Date of birth:	January 14, 1956
Place of birth:	St-Jean-de-Québec

Education

B.Sc. in Food Science, 1979.
Département de sciences et technologie des aliments, Université Laval, Québec.

Ph. D. degree in Food Engineering, 1986.
Department of Food Science, University of Alberta, Edmonton, Alberta.

Awards received

1977	Empire Dehydrated Products Co. Scholarship.
1977-78	Department of Industry, Trade and Commerce, Canadian Food Industry Scholarship.
1978-79	Department of Industry, Trade and Commerce, Canadian Food Industry Scholarship.
1980-81	Postgraduate Scholarship, Natural Sciences and Engineering Research Council of Canada.
1981-82	Postgraduate Scholarship, Natural Sciences and Engineering Research Council of Canada.
1983-84	Farming for the Future - Graduate Student Research Program, Alberta Heritage Foundation.

Professional experience

1978	Research assistant in Food Microbiology, in the area of production of baker yeasts on fermented whey. Département de sciences et technologie des aliments, Université Laval, Québec.
1979	Research and development, in the area of stability of orange juice during storage. Département de sciences et technologie des aliments, Université Laval, Québec, et Lassonde et Fils, Rougemont.
1980-81	Teaching assistant; organisation and coordination of the following laboratories: "Introduction to Food Engineering", and "Unit Operations in Food Processing 1". Department of Food Science, University of Alberta, Edmonton.
1981-82	Teaching assistant; organisation and coordination of the following laboratory: "Heat and Mass Transfer in Food Engineering". Department of Food Science, University of Alberta, Edmonton.
1982-83	Lecturer for the following courses: "Introduction to Food Engineering", and "Unit Operations in Food Processing 1". Department of Food Science, University of Alberta, Edmonton.
1982	Appointed as Food Engineer with Agriculture Canada.

Professional affiliations

Member of the following associations:

American Society for Quality Control (ASQC).

Canadian Institute of Food Science and Technology (CIFST).

Institute of Food Technologists (IFT).

The Society for Computer Simulation (SCS).

Publications and presentations

C.J. Toupin et J. Rivard. *Enrichissement de laits à faible teneur en gras par addition de lactosérum.* Internal report, Département de sciences et technologie des aliments, Université Laval, Québec.

C.J. Toupin, M. Le Maguer, P. Jelen and R.I.W. Greig. *Water Sorption in Heat-denatured Whey Proteins Powders.* Paper presented at the 40th annual IFT meeting. New-Orleans, Louisiana, June 8-11, 1980.

C.J. Toupin and M. Le Maguer. *A New Statistical Method for the Evaluation of BET Constants from Water Sorption Isotherms of Whey Proteins.* Paper presented at the 35th annual meeting of the Pacific Northwest Region of the American Society of Agricultural Engineers. Great Falls, Montana, October 1-3, 1980.

C.J. Toupin and M. Le Maguer. *A New Statistical Approach for the Evaluation of BET Constants from Water Sorption Isotherms.* Paper presented at the 24th annual conference of the Canadian Institute of Food Science and Technology. Winnipeg, Manitoba, June 21-24, 1981.

C.J. Toupin and M. Le Maguer. *Mass Transfer in Cellular Foodstuffs in Direct Contact with Aqueous Freezant.* Paper presented at the 36th annual meeting of the Pacific Northwest Region of the American Society of Agricultural Engineers. Edmonton, Alberta, September 2-4, 1981.

C.J. Toupin and M. Le Maguer. *Mass Transfer in Cellular Foodstuffs in Contact with Aqueous Freezants. Theoretical Model and Simulation.* Paper presented at the 25th annual conference of the Canadian Institute of Food Science and Technology. Montréal, Québec, May 30 - June 3, 1982.

C.J. Toupin, M. Le Maguer and J.R. McGregor. *The Evaluation of BET Constants from Sorption Isotherms Data.* Lebens. Wiss. + Technol. 16: 153-156 (1983).

C.J. Toupin and M. Le Maguer. *Application of Irreversible Thermodynamics to the Prediction of Mass Transfer in Cellular Foodstuffs*. In: *Engineering and Food*. Volume 1. *Engineering Sciences in the Food Industry*. B.M. McKenna, editor. Elsevier Applied Science Publ. London. 1984. pp. 41-48.